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# PROCEEDINGS

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SERIES B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

VOL. LXXXIV.

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# CONTENTS.

## SERIES B. VOL. LXXXIV.

No. B 568.—July 3, 1911.

PAGE

The Causes of Absorption of Oxygen by the Lungs in Man. (Preliminary Communication.) By C. Gordon Douglas, B.M., Fellow of St. John's College, and J. S. Haldane, M.D., LL.D., F.R.S., Fellow of New College, Oxford ......... 1

On the Inter-relations of Genetic Factors. By W. Bateson, F.R.S., and R. C. Punnett, Professor of Biology in the University of Cambridge ........................................ 3

A Case of Gametic Coupling in Pisum. By Philippe de Vilmorin and W. Bateson, F.R.S. ........................................ 9

On Gametic Coupling and Repulsion in *Primula sinensis*. By R. P. Gregory, M.A., Fellow of St. John's College, Cambridge, University Lecturer in Botany. Communicated by W. Bateson, F.R.S. ........................................ 12

The Action of Animal Extracts on Milk Secretion. By E. A. Schäfer, F.R.S., and K. Mackenzie, M.B., Ch.B. ........................................ 16

Inbreeding in a Stable Simple Mendelian Population with Special Reference to Cousin Marriage. By S. M. Jacob, I.C.S., Biometric Laboratory, University College, London. Communicated by Prof. Karl Pearson, F.R.S. .................. 23

Cancerous Ancestry and the Incidence of Cancer in Mice. By J. A. Murray, M.D., B.Sc., Imperial Cancer Research Fund. Communicated by Prof. J. Rose Bradford, Sec. R.S. ........................................ 42

Immunisation by Means of Bacterial Endotoxins. By R. Tanner Hewlett, M.D. Communicated by Prof. W. D. Halliburton, F.R.S. ........................................ 49

On a Method of Disintegrating Bacterial and other Organic Cells. By J. E. Barnard and R. T. Hewlett. Communicated by Prof. W. D. Halliburton, F.R.S. (Plate 1) ........................................ 57

Motor Localisation in the Brain of the Gibbon, correlated with a Histological Examination. By F. W. Mott, Edgar Schuster, and C. S. Sherrington. Communicated by Prof. C. S. Sherrington, F.R.S. ........................................ 67

Experiments on the Restoration of Paralysed Muscles by means of Nerve Anastomosis. By Robert Kennedy, M.A., M.D., D.Sc. Communicated by Prof. J. G. McKendrick, F.R.S. (Abstract) ........................................ 75

A Preliminary Note on the Extrusion of Granules by Trypanosomes. By W. B. Fry, Captain R.A.M.C. Communicated by H. G. Plimmer, F.R.S. ........................................ 79

No. B 569.—July 20, 1911.

PAGE

The Properties of Colloidal Systems.—II. On Adsorption as Preliminary to Chemical Reaction. By W. M. Bayliss, F.R.S., Institute of Physiology, University College, London ..... 81

On the Distribution and Action of Soluble Substances in Frogs Deprived of their Circulatory Apparatus. By S. J. Meltzer, New York. Communicated by Prof. A. R. Cushny, F.R.S. ..... 98

The Mechanism of Carbon Assimilation: Part III. By Francis L. Usher and J. H. Priestley. Communicated by Dr. M. W. Travers, F.R.S. ..... 101

Transmission of Amakebe by means of *Rhipicephalus appendiculatus*, the Brown Tick. By Dr. A. Theiler, C.M.G., Pretoria. Communicated by Colonel Sir D. Bruce, C.B., F.R.S. ..... 112

The Discrimination of Colour. By F. W. Edridge-Green, M.D., F.R.C.S., Beit Medical Research Fellow. Communicated by Prof. W. M. Bayliss, F.R.S. ..... 116

Note on the Sensibility of the Eye to Variations of Wave-length. By W. Watson, D.Sc., F.R.S. ..... 118

On the Direct Guaiacum Reaction given by Plant Extracts. By Miss M. Wheldale, Fellow of Newnham College, Cambridge. Communicated by W. Bateson, F.R.S. ..... 121

The Action of Radium Radiations upon Some of the Main Constituents of Normal Blood. By Helen Chambers, M.D., and S. Russ, D.Sc., Beit Memorial Research Fellow. Communicated by Dr. J. R. Bradford, Sec. R.S. ..... 124

On a New Method of Estimating the Aperture of Stomata. By Francis Darwin, F.R.S., and D. F. M. Pertz ..... 136

No. B 570.—August 18, 1911.

The Experimental Transmission of Goitre from Man to Animals. By Robert McCarrison, M.D., M.R.C.P. (Lond.), Captain, Indian Medical Service; Agency Surgeon, Gilgit, Kashmere. Communicated by Major Ronald Ross, C.B., F.R.S. (Plate 1*) ..... 155

The Pathogenic Agent in a Case of Human Trypanosomiasis in Nyasaland. By Hugh S. Stannus and Warrington Yorke. Communicated by Major R. Ross, F.R.S. (Plate 2) ..... 156

Note on Developmental Forms of *Trypanosoma brucei* (*pecaudi*) in the Internal Organs, Axillary Glands, and Bone-marrow of the Gerbil (*Gerbillus pygargus*). By George Buchanan, Senior Laboratory Assistant, Wellcome Tropical Research Laboratories, Gordon College, Khartoum. Communicated by Col. Sir David Bruce, C.B., F.R.S. (Plate 3) ..... 161

A Contribution to our Knowledge of the Protozoa of the Soil. By T. Goodey, M.Sc. (Birm.), Mackinnon Student of the Royal Society, Rothamsted Experimental Station. Communicated by A. D. Hall, F.R.S. (Plate 4) ..... 165

The Morphology of *Trypanosoma evansi* (*Steel*). By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service. (Plate 5) ..... 181

PAGE

On the Action of Senecio Alkaloids and the Causation of Hepatic Cirrhosis in Cattle. (Preliminary Note.) By Arthur R. Cushny, F.R.S. .................................... 188

The Viability of Human Carcinoma in Animals. By Major C. L. Williams, M.D., I.M.S. ret. Communicated by Prof. C. S. Sherrington, F.R.S. ..................... 191

On Ceratopora, the Type of a New Family of Alcyonaria. By Sidney J. Hickson, F.R.S., Professor of Zoology in the University of Manchester. (Plate 6) ...... 195

On Reflex Inhibition of the Knee Flexor. By C. S. Sherrington, F.R.S., and S. C. M. Sowton .................................................................................. 201

No. B 571.—September 14, 1911.

The Structure and Physiological Significance of the Root-nodules of *Myrica gale.* By W. B. Bottomley, M.A., Professor of Botany in King's College, London. Communicated by Prof. J. Reynolds Green, F.R.S. ..................................... 215

Note on the Surface Electric Charges of Living Cells. By W. B. Hardy, F.R.S., and H. W. Harvey .......................................................................... 217

The Origin of Osmotic Effects. IV.—Note on the Differential Septa in Plants with reference to the Translocation of Nutritive Materials. By Henry E. Armstrong, F.R.S., and E. Frankland Armstrong.......................................................... 226

The Properties of Colloidal Systems. III.—The Osmotic Pressure of Electrolytically Dissociated Colloids. By W. M. Bayliss, F.R.S., Institute of Physiology, University College, London .................................................. 229

On the Fate of Red Blood Corpuscles when Injected into the Circulation of an Animal of the same Species; with a New Method for the Determination of the Total Volume of the Blood. By Charles Todd, M.D., Bacteriologist, Egyptian Government; and R. G. White, M.B., Director, Serum Institute, Cairo. Communicated by Dr. C. J. Martin, F.R.S. ...................................... 255

Electrical Effects accompanying the Decomposition of Organic Compounds. By M. C. Potter, Sc.D., M.A., Professor of Botany in the University of Durham. Communicated by Dr. A. D. Waller, F.R.S. ............................................ 260

Fractional Withdrawal of Complement and Amboceptor by Means of Antigen. (Preliminary Note.) By J. O. Wakelin Barratt, M.D., D.Sc. Lond., Director of Cancer Research Laboratory, University of Liverpool (Mrs. Sutton Timmis Memorial). Communicated by Prof. C. S. Sherrington, F.R.S. ...................... 277

No. B 572.—December 8, 1911.

The Influence of Ionised Air on Bacteria. By W. M. Thornton, D.Sc., D.Eng., Professor of Electrical Engineering at Armstrong College, Newcastle-upon-Tyne. Communicated by Sir Oliver Lodge, F.R.S. (Plates 7—12)............... 280

The Permeability of the Yeast-Cell. By Sydney G. Paine, Research Scholar in the Biochemical Department, Lister Institute, London. Communicated by Arthur Harden, F.R.S. ........................................................................... 289

PAGE

The Intrinsic Factors in the Act of Progression in the Mammal. By T. Graham Brown. Communicated by Prof. C. S. Sherrington, F.R.S. .......... 308

An Inquiry into the Influence of the Constituents of a Bacterial Emulsion on the Opsonic Index. By A. F. Hayden, M.B., B.S., F.R.C.S., I.M.S., and W. Parry Morgan, M.A., B.Sc., M.B. Communicated by Sir A. E. Wright, F.R.S. .......... 320

The Morphology of *Trypanosoma gambiense* (Dutton). By Colonel Sir David Bruce, C.B., F.R.S., A.M.S. (Plate 13) .......... 327

The Behaviour of the Infusorian Micronucleus in Regeneration. By Kenneth R. Lewin, B.A., Senior Scholar and Coutts Trotter Student of Trinity College, Cambridge. Communicated by Prof. J. S. Gardiner, F.R.S. .......... 332

The Refractive Indices of the Eye Media of some Australian Animals. By Judah Leon Jona, D.Sc., M.B., B.S. Communicated by Prof. J. N. Langley, F.R.S. .... 345

No. B 573.—December 28, 1911.

Ventilation of the Lung during Chloroform Narcosis. By G. A. Buckmaster and J. A. Gardner. Communicated by Dr. A. D. Waller, F.R.S. .......... 347

Factors in the Interpretation of the Inhibitive and Fixation Serum Reactions in Pulmonary Tuberculosis. By Alfred H. Caulfeild, M.B. Communicated by Prof. T. G. Brodie, F.R.S. .......... 373

Preliminary Report upon the Injection of Rabbits with Protein-free (Tuberculo-) Antigen and Antigen-Serum Mixtures. By Alfred H. Caulfeild, M.B. Communicated by Professor T. G. Brodie, F.R.S. .......... 390

Studies in Heredity. I.—The Effects of Crossing the Sea-urchins *Echinus esculentus* and *Echinocardium cordatum*. By Prof. E. W. MacBride, F.R.S., Imperial College of Science and Technology, South Kensington .......... 394

The Physiological Influence of Ozone. By Leonard Hill, F.R.S., and Martin Flack 404

On the Factors Concerned in Agglutination. By H. R. Dean, M.A., M.B., Ch.B., M.R.C.P., Assistant Bacteriologist, Lister Institute, London. Communicated by Dr. C. J. Martin, F.R.S. .......... 416

No. B 574.—February 14, 1912.

Address of the President, Sir Archibald Geikie, K.C.B., at the Anniversary Meeting on November 30, 1911 .......... 435

Action of Dissolved Substances upon the Autofermentation of Yeast. By Arthur Harden, F.R.S., and Sydney G. Paine .......... 448

Further Experiments upon the Blood Volume of Mammals and its Relation to the Surface Area of the Body. By Georges Dreyer, M.A., M.D., Professor of Pathology in the University of Oxford, and William Ray, M.B., B.Sc., Philip Walker Student in the University of Oxford. Communicated by Prof. Francis Gotch, F.R.S. (Abstract) .......... 460

The Origin and Destiny of Cholesterol in the Animal Organism. Part VIII.—On the Cholesterol Content of the Liver of Rabbits under Various Diets and during Inanition. By G. W. Ellis and J. A. Gardner. Communicated by Dr. A. D. Waller, F.R.S. .......... 461

PAGE

Herbage Studies. I.—*Lotus corniculatus*, a Cyanophoric Plant. By Henry E. Armstrong, F.R.S., E. Frankland Armstrong and Edward Horton ................ 471

Antelope Infected with *Trypanosoma gambiense*. By Captain A. D. Fraser, R.A.M.C., and Dr. H. L. Duke. Communicated by Sir John Bradford, Sec. R.S. ........................................................................................ 484

The Bacterial Production of Acetylmethylcarbinol and 2.3-Butylene Glycol from Various Substances. By Arthur Harden, F.R.S., and Dorothy Norris ......... 492

The Chemical Action of *Bacillus cloacæ* (Jordan) on Glucose and Mannitol. By James Thompson. Communicated by Arthur Harden, F.R.S. ...................... 500

*Herpetomonas pediculi*, nov. spec., Parasitic in the Alimentary Tract of *Pediculus vestimenti*, the Human Body Louse. By H. B. Fantham, D.Sc., B.A., Christ's College, Cambridge, and Liverpool School of Tropical Medicine. Communicated by Prof. Sir Ronald Ross, K.C.B., F.R.S. (Plate 14) ......................... 505

No. B 575.—March 12, 1912.

A Method for Isolating and Cultivating the *Mycobacterium enteritidis chronicæ pseudotuberculosæ bovis*, Jöhne, and Some Experiments on the Preparation of a Diagnostic Vaccine for Pseudo-tuberculous Enteritis of Bovines. By F. W. Twort, M.R.C.S., L.R.C.P. Lond., and G. L. Y. Ingram, M.R.C.V.S. Communicated by Leonard Hill, M.B., F.R.S. ....................................................... 517

On the Fossil Flora of the Forest of Dean Coalfield (Gloucestershire), and the Relationships of the Coalfields of the West of England and South Wales. By E. A. Newell Arber, M.A., F.L.S., F.G.S., Trinity College, Cambridge, University Demonstrator in Palæobotany. Communicated by Prof. T. McKenny Hughes, F.R.S. (Abstract) ..................................................... 543

Simultaneous Colour Contrast. By F. W. Edridge-Green, M.D., F.R.C.S., Beit Memorial Research Fellow. Communicated by Prof. E. Starling, F.R.S. ...... 546

An Alleged Specific Instance of the Transmission of Acquired Characters.—Investigation and Criticism. By T. Graham Brown (Carnegie Fellow). Communicated by Prof. C. S. Sherrington, F.R.S. .................................................... 555

Note on *Astrosclera willeyana* Lister. By R. Kirkpatrick. Communicated by S. F. Harmer, F.R.S. ........................................................................... 579

Erratum ........................................................................................... 580

OBITUARY NOTICES OF FELLOWS DECEASED.

Sydney Ringer; Sir Rubert Boyce; Sir Francis Galton; John Hughlings Jackson; John Beddoe ................................................................ i—xxvii

Index ............................................................................................. xxix

PROCEEDINGS OF

THE ROYAL SOCIETY.

*Section B.—Biological Sciences.*

# *The Causes of Absorption of Oxygen by the Lungs in Man. (Preliminary Communication.)*

By C. Gordon Douglas, B.M., Fellow of St. John's College, and
J. S. Haldane, M.D., LL.D., F.R.S., Fellow of New College, Oxford.

(Received February 23,—Read March 23, 1911.)

In a previous communication* we gave a short account of experiments on mice, showing, by the carbon monoxide method of determining the partial pressure of oxygen in the arterial blood, that when want of oxygen is produced by administering to these animals a relatively high percentage of carbon monoxide in the air breathed, active secretion of oxygen inwards through the lung epithelium occurs, although in resting animals the passage inwards of oxygen is apparently due to nothing but simple diffusion.

It was very desirable to extend these experiments to man, as the main chemical factors in respiration can be much more satisfactorily investigated in man than in lower animals, and we have now succeeded in obtaining a number of results in man. In the case of a man it would require many hours to complete an experiment made by exactly the same method as was employed for mice and rabbits. We have therefore adopted the plan of first rapidly administering sufficient carbon monoxide to bring the blood to the required degree of saturation with the gas, and then allowing the subject to breathe into a closed space of about 15 litres, in the air of which the carbon dioxide is absorbed and the oxygen kept constant on the principle of Regnault and Reiset. The partial pressure of carbon monoxide in this air becomes equal in a few minutes to that in the blood, and part of this air is thereafter used for saturating the sample of the subject's blood, the principle of the experiment being otherwise the same as in the experiments on animals.

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* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 331.

The following results have been obtained:—

(1) During rest under normal conditions, and provided that the blood is not more than about 25 per cent. saturated with carbon monoxide, the partial pressure of oxygen in the arterial blood is practically identical with that in the alveolar air. This result accords completely with the theory that under these conditions the absorption of oxygen is by diffusion alone.

(2) When the percentage of oxygen in the inspired air is lowered sufficiently (or the saturation of the blood with carbon monoxide is increased sufficiently) to cause appreciable symptoms of want of oxygen, the partial pressure of oxygen in the arterial blood becomes very considerably higher than in the alveolar air. Active secretion of oxygen inwards is therefore occurring, as was formerly concluded by Haldane and Lorrain Smith from experiments on mice. We find, however, that Haldane and Lorrain Smith's results require a considerable and at present somewhat uncertain correction.

(3) During muscular work also, unless the work was of a comparatively gentle kind, a similar result was obtained, and muscular work with the inspired air poor in oxygen seemed to produce a specially striking effect.

Taken together, the results indicate that the lung epithelium is excited directly or indirectly to active secretion of oxygen inwards by products of metabolism proceeding from the muscles and other tissues when their oxygen supply is insufficient to meet ordinary requirements. That such insufficiency actually occurs during muscular work, and when air with a low partial pressure of oxygen is breathed, has already been shown.* These results are of special interest in connection with the phenomena of adaptation to very high altitudes, and throw a new light on the physiology of mountain climbing and balloon ascents, and of ordinary muscular work.

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* Geppert and Zuntz, 'Pflüger's Archiv,' 1888, vol. 42, p. 189; Boycott and Haldane, 'Journ. of Physiol.,' 1908, vol. 37, p. 355; Ogier Ward, *ibid.*, vol. 37, p. 378; Haldane and Poulton, *ibid.*, vol. 37, p. 390; Douglas and Haldane, *ibid.*, 1909, vol. 38, p. 420; Ryffel, *ibid.*, vol. 38, p. 29; Boycott and Chisolm, 'Biochemical Journ.,' 1910.

# *On the Inter-relations of Genetic Factors.*

By W. Bateson, F.R.S., and R. C. Punnett, Professor of Biology in the University of Cambridge.

(Received March 2,—Read March 30, 1911.)

The nature and bearing of the observations to be recorded in this paper will best be explained by tracing the steps by which they have been reached.

Early in the investigation of heredity in the sweet pea it was observed that when plants were heterozygous for two separate pairs of allelomorphs the distribution of the factors concerned was in certain cases disturbed in definite ways, such that particular combinations occurred in the gametes with greater frequency than others.

(1) The first case noticed was that of $F_1$ plants heterozygous for *blue* and *red* colour, and for *long* and *round* pollen. In the $F_2$ generation all possible combinations were represented, but the blues were for the most part long-pollened and the reds were for the most part round-pollened.

(2) The next case observed was that of $F_1$ plants heterozygous for *dark* and *light* axils on the one hand, and for *fertile* and *sterile* anthers on the other. In this $F_2$ also all combinations occurred, but nearly all the dark-axil plants had fertile anthers, while nearly all the light-axilled plants had sterile anthers.

(3) The next step was made by a study of the $F_2$ from plants heterozygous for *blue* and *red* flowers and for *erect* and *hooded* standards. Here it was found that one of the possible combinations did not exist in $F_2$, for though the blues might be either erect or hooded, the reds were *all* erect.

Examining these occurrences in the light of the presence-and-absence theory, it was clear that the phenomenon presented by cases (1) and (2) was entirely distinct from that presented by case (3). For whereas in (1) and (2) there was excess of gametes bearing the two factors over those bearing one or the other alone, the condition produced in (3) could only be obtained by a distribution such that no gamete could carry *both* positive factors. We were therefore led to recognise—

A. A system of partial *coupling* under which two factors are generally associated.

B. A system of complete *repulsion* (or as we have sometimes called it, "spurious allelomorphism") under which two factors are never associated in the same gamete.

The partial coupling was next shown to be approximately in case (1)

7BL : 1Bl : 1bL : 7bl,

where B is blue and L is long pollen;*

and in case (2) to be

15DF : 1Df : 1dF : 15df,

where D is dark axil and F is fertile anthers.

(4) At this stage, investigation of the properties of the exceptional members of the $F_2$ series was begun. In particular, the combination dark axil with sterile anthers (Df) was crossed with a light-axilled plant having normal anthers (dF). The $F_2$ generation from this cross was, to our surprise, a series in which *all the sterile plants had dark axils.* Here, therefore, there had been a repulsion between the same two factors which had been coupled in case (2).

In considering what could have determined this difference in behaviour, it seemed possible that the distinction might have been due to the way in which the factors had been combined in the original parents, for we knew that in the cases where coupling had resulted, the two dominant factors had been introduced together from the same parent, whereas in this new case one had come from each parent.

For several years this conjecture has been made the subject of elaborate tests, and its correctness has now been completely substantiated in several examples. Expressed in a general form, the conclusion to which we have been led is that if A, a, and B, b, are two allelomorphic pairs subject to coupling and repulsion, the factors A and B will repel each other in the gametogenesis of the double heterozygote resulting from the union

Ab × aB,

but will be coupled in the gametogenesis of the double heterozygote resulting from the union

AB × ab.

The $F_1$ heterozygote is ostensibly identical in the two cases, but its offspring reveals the distinction. We have as yet no probable surmise to offer as to the essential nature of this distinction, and all that can yet be said is that in these special cases the distribution of the characters in the heterozygote is affected by the distribution in the original pure parents.

In $F_2$, from a system in which A and B are coupled, almost all the offspring in the form AaBb will be again built up from AB and ab gametes, so that they will again exhibit coupling; but a very small proportion will be formed from the comparatively rare gametes aB and Ab. Such heterozygotes will probably show repulsion in their gametogenesis. They must, however, be so rare (only 2 in 256, for example, from the system 7AB : 1aB : 1Ab : 7ab) that it is almost hopeless to look for them in practice.

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* There are indications that this distribution may be liable to disturbance by other factors in a way not yet understood ('Reports Evol. Committee,' IV, pp. 11—13).

We know, moreover, that these phenomena are not peculiar to the sweet pea, but that they must exemplify widespread principles of genetic physiology. Repulsion has been found between the factor for femaleness and several factors of various kinds in animals—*e.g.*, in *Abraxas grossulariata*; in the canary; in the fowl for at least three factors—*i.e.* (1) the factor which inhibits the development of the peculiar mesoblastic pigment of the Silky, (2) the dominant "silver" of Assendelvers (Hagedoorn) and of Sebrights (ourselves, unpublished), (3) the barring factor of Plymouth Rocks (Spillman; Pearl). Coupling till recently had been observed in the sweet pea only. Now we have the additional examples published simultaneously with this note, namely, tendrils and round seed in Pisum (de Vilmorin and Bateson), and short style and magenta colour in *Primula sinensis* (Gregory). In addition to these cases of coupling, Gregory also contributes a new example of repulsion, between green stigma and the factor which diminishes the stem-colour. There is thus good reason to believe that these phenomena are of no restricted occurrence in nature.

In work already published we have shown that coupling occurs according to the systems

7AB : 1aB : 1Ab : 7 ab

and 15AB : 1aB : 1Ab : 15ab.

Such systems pointed to the existence of others which could be given by the expression

$$3n^2-(2n-1) : 2n-1 : 2n-1 : n^2-(2n-1),$$

where $n$ is half the number of gametes needed to express the whole system.

Two more of the systems thus contemplated as possibilities have been discovered. The cases now stand thus:—

3 : 1. No case yet known.
7 : 1. *Sweet Pea.* Blue factor and long pollen.
7 : 1. *Primula sinensis.* Magenta colour and short style.
15 : 1. *Sweet Pea.* Fertile anthers and dark axils.
31 : 1. No case yet known.
63 : 1. *Pisum.* Development of tendrils and round seed.
127 : 1. *Sweet Pea.* Blue factor and erect standard.

For all of these except the Pisum case (as yet untried) repulsion is also proved to occur. We know also that repulsion occurs between long pollen and the erect standard in families where blues are not present, but hitherto we have not had an opportunity of determining the system of coupling followed by this pair of factors.

Several curious and important lines of inquiry are thus opened up. As to the actual meaning or nature of coupling or repulsion there is no clue. The fact, however, that the mode in which factors are combined in the original parents can influence the distribution of the factors among the gametes of $F_1$ introduces a new conception into genetic physiology. Reciprocal matings give identical results, so no mere question of maternal influence is involved.

In attempting to form any conception of what actually happens in coupling or repulsion, and of the cause which determines that the one phenomenon or the other shall occur, we are met at once by the difficulty that we do not yet know how or when the system 1 AB : 1 aB : 1 Ab : 1 ab, which we regard as the normal distribution for two pairs of allelomorphs, is produced. There is as yet no proof that the segregation of both pairs of factors occurs at one division, or that that division is one of those which we regard as specially concerned in maturation. Now that we know of a series involving as many as 256 terms $(127+1+1+127)$ it is most difficult to conceive that such a system can be produced in the maturation-divisions of the ovarian tissue of such a plant as a sweet pea. We may well be tempted to look much earlier in the developmental processes for the establishment of these differentiations, and it is not impossible that they may be established as early as the embryonic constitution of the sub-epidermal layer itself. As is known, this layer is—in most higher plants, at least—the exclusive source of the germ-cells, a fact which leads to those remarkable consequences which Baur has discovered in the genetics of variegated plants. Remote as this possibility admittedly is, in a problem of such extreme difficulty even improbable suggestions are worthy of consideration.

If we knew how the normal distribution, 1 AB, 1 aB, 1 Ab, 1 ab, is brought about we might surmise by what modification the other distributions are created. As it is, we can only say that in repulsion the heterozygote AaBb gives off germ-cells of two types, Ab and aB, whereas in a coupled system there are four types, AB, Ab, aB, ab, the two terms AB and ab being represented 7 times, 15 times, etc. One step further may perhaps be gained by arranging the symbols so as to represent the combinations more accurately to the eye—

| | |
|---|---|
| 1. Ab × aB. | 2. AB × ab. |
| \| | \| |
| Ab . aB. | AB . ab. |

The heterozygote Ab . aB forms only two types of gametes, and the heterozygote AB . ab gives the coupled series of four types. Since the same factors are involved in both cases it looks possible that the difference in behaviour may be a consequence of the difference in the geometrical positions of the factors relative to the planes of some critical division or divisions in the two cases. There may, in fact, be a difference of polarity between the two kinds of heterozygote.

The increase in number of the two types of cell, AB and ab, may be reached by proliferation of the two primordial cells of those two types. It may further be remarked that though the numbers characteristic of coupled systems cannot be produced by simple dichotomies, they can readily be represented as produced by a series of periclinal and anticlinal divisions. For example if $AB^1$ by periclinal division give off $AB^2$, and this by anticlinal division become two cells, which again divide periclinally and anticlinally, seven cells AB are formed; by repetition of the same processes 15 are formed, and so on.

*Systems of three Factors.*—From the list given above it will be seen that in the sweet pea we know two distinct factors, viz., erect standard and long pollen, which may be severally coupled with a third factor, that for blue colour. Here, therefore, we meet a system of inter-relationship between *three* pairs, and special interest must attach to a determination of the genetic properties of plants heterozygous for all three. (The distribution of the factors for fertile anthers and dark axils, so far as evidence goes, is independent of this system of three pairs, so that, for the present, fertility of anthers and axil-colour can be left out of account in a consideration of the triple system.)

A plant heterozygous for B (blue), L (long pollen), and E (erect standard), can be made by any of four possible combinations.

(1) EBL × ebl.
(2) EBl × ebL.
(3) Ebl × eBL.
(4) eBl × EbL.

All these various types of combinations are now either made or being made, but as yet we are only able to give the result in the case of No. 3. In it B and E repel, and B is coupled with L on the 7 : 1 system. The coupling of B with L, since they come in together, may seem to be what the general trend of the evidence leads us to expect, but the fact that E is repelled by B rather than by L is worthy of special notice, for we know that E and L repel each other when B is not present. It suggests that there must be an "order of precedence" among the factors composing such a system, and the suggestion is plausible that this order will follow the grade of coupling in which the factors are accustomed to be linked.

It will be observed that, given a system under which a pair of factors are coupled, it is possible to produce the system under which the same pair repel each other. For all that is necessary is to breed together the rarer terms of the coupled series, viz., Ab and aB.

From the repelling system, on the contrary, in the absence of a fresh variation, we have no obvious way of constructing the coupled system. This consideration has an obvious application to those cases in which *sex* operates as a repelling factor. In the fowl, the canary, and *Abraxas grossulariata*, femaleness thus acts as a repelling factor against various elements determining pigmentation; and our experience of the plants leads us to suppose that if the factors involved could be built up in the right combinations, femaleness might be coupled with the factors it now repels.

Extraordinary consequences, both to the distribution of the sexes, to the distribution of factors between them, and perhaps to the causation of fertility, must be anticipated if this condition could be fulfilled. There may be an indirect way of actually accomplishing these results. For, seeing that sex in the fowl acts as a repeller of at least three other factors, when birds are built up so as to be heterozygous for several of these, some of them may be found able to take precedence of the others in such a way as to annul the present repulsions, with subsequent coupling as a consequence.

# *A Case of Gametic Coupling in Pisum.*

By PHILIPPE DE VILMORIN and W. BATESON, F.R.S.

(Received March 2,—Read March 30, 1911.)

For some years past a variety of culinary peas has been grown at Verrières-le-Buisson, remarkable for the fact that it has no tendrils, each of the normal tendrils being represented by a leaflet. The figure shows the appearance of the leaves of this variety, with leaves of normal plants for comparison.

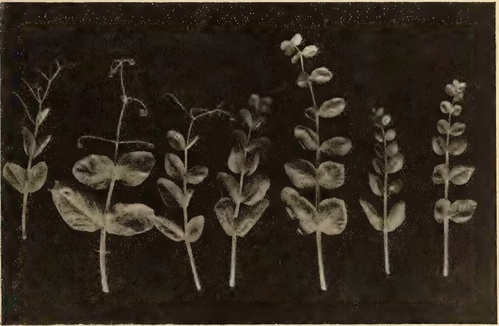

FIG. 1.—The four right-hand leaves are from "Acacia" peas; the three left-hand leaves are from normal plants. The figure on extreme left shows a leaflet with a tendril opposite to it. Such asymmetries are common in the normal types.

These "Acacia" peas, as they are called, breed perfectly true. Their origin is unknown. The variety has wrinkled seeds.

Crosses were made at Verrières between the Acacias and a variety having normal tendrils and round seeds. The tendrilled character was fully dominant in $F_1$, which, of course, bore both round and wrinkled seeds. When these seeds were separately sown it was observed that the round seeds gave rise almost exclusively to tendrilled plants, and the wrinkled seeds almost exclusively to Acacias, though there were a few exceptions each way.

As the case was almost certainly one of gametic coupling between roundness of seed, and tendrils, the problem was clearly worth minute investigation, and a quantity of material was transferred to the John Innes Horticultural Institution, Merton, Surrey, for this purpose. The offspring of two $F_2$ plants, heterozygous in both respects, were, in particular, the subject of study.

Unfortunately, the distinction between rounds and wrinkleds was in these families not perfectly sharp, and much irregular pitting occurred. When the plants grown from these seeds were harvested it was found that errors in sorting had been made both ways, one seed having been sown for a round which gave rise to a plant with exclusively wrinkled seeds, and two seeds which had been sown as wrinkled proved to have been heterozygous rounds. The earlier counts had therefore to be rejected, and in order to obtain perfectly reliable numbers it was clearly necessary that the nature of the starch in each seed should be separately determined for each seed before it was sown. As Gregory* had observed, by microscopical examination of the starch grains, this discrimination may be made without difficulty.

The microscopical test was applied by Miss C. Pellew, Minor Student of the Institution, to $F_4$ seeds of heterozygous plants on a considerable scale, and the seeds were then sown. Only a fragment of a cotyledon need be removed for testing, and the seeds germinated perfectly after the operation. The results were as follows:—

| | Round. | | Wrinkled. | |
|---|---|---|---|---|
| | Tendrilled. | Acacia. | Tendrilled. | Acacia. |
| Observed | 319 | 4 | 3 | 123 |
| Calculated on a coupling of 63 : 1 | *333* | *3·4* | *3·4* | *109* |

These figures leave no reasonable doubt that the system of gametic coupling followed in this case is

63 TR : 1 Tr : 1 tR : 63 tr,

where T is tendrilled, and R is round seed. This gametic system gives the zygotic ratio

12161 TR : 127 Tr : 127 tR : 3969 tr = 16384,

from which the above calculation is made. This particular system has not been hitherto encountered, but it is, of course, one of those contemplated by the general expression for coupled systems.

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* 'New Phytologist,' 1903, vol. 2, p. 226.

There is no difficulty in distinguishing the tendrilled from the acacia plants when six to seven leaves are developed, but in some of the tendrilled—doubtless the heterozygotes—the apical tendril is sometimes strap-shaped, especially in the youngest tendril-bearing leaves.

On one occasion at Verrières, Acacias came in a strain of Sandar's Marrow. Though natural cross-fertilisation is extremely rare among peas (much rarer than in sweet peas) we can hardly doubt that these Acacias were recessives extracted after an accidental cross with the pure strain growing in the same garden. Both among various peas grown at Verrières, at Reading, and at Grantchester, a few unquestionable examples of crossing have been observed since critical attention has been devoted to the study of heredity in peas. The crossing is probably effected by visits of *Megachile* to flowers in which for some reason their own pollen has been inoperative.

In the case of the derivatives from the Sandar's Marrow strain the occurrence of strap-shaped tendrils, presumably on the heterozygotes, has been often observed, and some plants have many such intermediate tendrils.

The original cross which gave coupling between T and R was in the form TR × tr. Experiments are now in progress for testing whether when a cross is made in the form Tr × tR the gametogenesis of $F_1$ will show repulsion of T from R, and on the analogy of what has been seen in sweet pea and in *Primula sinensis* this result may be confidently anticipated.

Whether any similar inter-relation exists between the tendril factor and factors other than that for round seed cannot be yet stated, but it is practically certain that the factors for yellow seed and for tall stem do not stand in any such special relation to it. The case is also interesting inasmuch as it is the first yet met with in which neither of the coupled factors is in any way concerned in determining pigmentation.

In conclusion it may be remarked that an identical "acacia" variety exists in the sweet pea, and its properties are also under investigation. In the sweet pea, however, there is no variety with truly wrinkled seed. The types with self-coloured lavender flowers have somewhat shrivelled seeds, but the starch of these is normal.

## *On Gametic Coupling and Repulsion in* Primula sinensis.

By R. P. Gregory, M.A., Fellow of St. John's College, Cambridge,
University Lecturer in Botany.

(Communicated by W. Bateson, F.R.S. Received March 2,—Read
March 30, 1911.)

In *Primula sinensis* the short style is dominant to the long style, and the magenta colour of the flower is dominant to the red colour.

Some years ago a series of experiments was made, in which a red short-styled race was mated with various long-styled plants carrying the factor for magenta colour. In $F_2$ from these crosses, only three kinds of offspring were obtained, namely: (1) magenta, short-styled; (2) magenta, long-styled; (3) red, short-styled. No red long-styled offspring were produced.* This result shows that, in the gametogenesis of the $F_1$, complete *repulsion* took place between the factors for the two dominant characters, magenta and short style.

In another series of crosses which have been made recently, a short-styled race carrying the magenta factor was crossed with two races of long-styled reds. The results obtained in $F_2$ show that, when the cross is made in this way, a *partial coupling* occurs between the factors for the two dominant characters.

One of the long-styled races used in these experiments was a red, with double flowers and green stigmas. In the two $F_2$-families raised from the crosses of this race with the short-styled race, the partial coupling observed is almost entirely certainly of the form 7 : 1 : 1 : 7.

The numbers obtained are :—

| | Magenta, short style. | Magenta, long style. | Red, short style, | Red, long style. | Total. |
|---|---|---|---|---|---|
| $F_1$ × self | 33 | 3 | 1 | 10 | 47 |
| *Expectation 7 : 1 : 1 : 7* | *32·5* | *2·8* | *2·8* | *9·0* | 47·1 |

In these two families there is no indication that either of the characters under consideration has any special inter-relation with any other character, the distribution of singles and doubles in the four types of offspring giving the normal ratio 9 : 3 : 3 : 1. The numbers obtained are :—

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* The experiments are described in detail in the 'Journ. Genetics,' 1911, vol. 1, No. 2.

| Magenta, single. | Magenta, double. | Red, single. | Red, double. | Total. |
|---|---|---|---|---|
| 27 | 9 | 9 | 2 | 47 |

| Short style, single. | Short style, double. | Long style, single. | Long style, double. | Total. |
|---|---|---|---|---|
| 24 | 10 | 12 | 1 | 47 |

The second long-styled race used was a dark red with red stigmas. In the families obtained from the crosses of this race with the short-styled race, the distribution of the offspring in the four classes is much less simple than in the preceding case; there is an excess of magentas and of short-styled plants, and the form which the partial coupling takes is not certain. The only family raised from the $F_1$ self-fertilised, containing as it did a large number of plants with colourless flowers, was too small to give any indication of the form of the coupling. The reciprocal crosses between the $F_1$ and the recessive parent race give results which are almost exactly intermediate between the expectation based on the series 7 : 1 : 1 : 7 and that based on the series 15 : 1 : 1 : 15. It is further to be noticed that in these families there is clear evidence that the factor for magenta is partially coupled, not only with the factor for short style, but also with a third factor, which has the effect of suppressing the development of pigment in the stigma, giving rise to the dominant green stigma. The partial coupling which is shown in this case is of a much lower type than that which obtains between magenta and short style, and, as in previous experiments,* does not exactly conform to any known series.

The fact that the magenta factor takes part in two systems of coupling, one of which is of an undetermined form, renders the results complex. Further data are required for their complete analysis, particularly in regard to the effect which the two systems of coupling, in combination, may have upon the distribution of the factors for short style and green stigma among the offspring. When the offspring are classified according to these two characters, the numbers observed are irregular, there being an excess of plants bearing the dominant characters.

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* 'Journ. Genetics,' 1911, vol. 1, No. 2.

The numbers which have been obtained are set out below:—

I.

| | Magenta, short style. | Magenta, long style. | Red, short style. | Red, long style. | Plants in which the magenta factor has no visible effect. | Total. |
|---|---|---|---|---|---|---|
| $F_1$ × self........................ | 5 | 1 | 1 | 2 | 21 | 30 |
| $F_1$ ♀ × red, long style, ♂ ... | 18 | 2 | 3 | 13 | 13 | 49 |
| Red, long style, ♀ × $F_1$ ♂ | 35 | 1 | 3 | 27 | 58 | 124 |
| Total: $F_1$ × red, long style | 53 | 3 | 6 | 40 | 71 | 173 |
| *Expectation: 7 : 1 : 1 : 7* | *44·6* | *6·4* | *6·4* | *44·6* | — | — |
| " *15 : 1 : 1 : 15* | *47·8* | *3·2* | *3·2* | *47·8* | — | — |

II.

| | Magenta, green stigma. | Magenta, red stigma. | Red, green stigma. | Red, red stigma. | Plants in which the magenta factor has no visible effect. | Total. |
|---|---|---|---|---|---|---|
| $F_1$ ♀ × red, red stigma, ♂ | 12 | 8 | 5 | 11 | 13 | 49 |
| Red, red stigma, ♀ × $F_1$ ♂ | 27 | 9 | 13 | 17 | 58 | 124 |
| Total ............... | 39 | 17 | 18 | 28 | 71 | 173 |

III.

| | Short style, green stigma. | Short style, red stigma. | Long style, green stigma. | Long style, red stigma. | Total. |
|---|---|---|---|---|---|
| $F_1$ × self ............................... | 16 | 7 | 4 | 3 | 30 |
| $F_1$ ♀ × long style, red stigma, ♂ | 19 | 10 | 11 | 9 | 49 |
| Long style, red stigma, ♀ × $F_1$ ♂ | 45 | 25 | 19 | 35 | 124 |
| Total: $F_1$ × long style, red stigma. | 64 | 35 | 30 | 44 | 173 |

Another instance of complete repulsion between two factors has been met with this year. The factors in question are: (1) a factor which effects the partial suppression of colour in the stem, and gives rise to the dominant light stems (pallifying factor), and (2) the factor, previously mentioned, which completely suppresses colour in the stigma.

The repulsion between these two factors was observed in the progeny of a cross in which a plant having red stems and red stigma was mated with a plant which was almost devoid of colour in the stem and had the dominant green stigma. The $F_2$ from this cross contained a long series of forms, and included plants having stems much darker in colour than those of the red-stemmed parent. The green-stemmed parent was therefore without the pallifying factor. Certain individuals of the $F_2$ were tested by self-fertilisation, and three of them, all having light red stems and green stigmas, gave $F_3$ families in which the complete repulsion between the pallifying factor and the factor for green stigma was shown, in the fact that none of the offspring with dark stems had red stigmas. The numbers obtained in the three families are shown below.

| | Light stem, green stigma. | Light stem, red stigma. | Dark stem, green stigma. | Dark stem, red stigma. | Total. |
|---|---|---|---|---|---|
| | 11 | 8 | 5 | 0 | 24 |
| | 95 | 40 | 45 | 0 | 180 |
| | 31 | 18 | 12 | 0 | 61 |
| Total ............ | 137 | 66 | 62 | 0 | 265 |
| *Expectation* ... | *132·5* | *66·25* | *66·25* | *0* | *265* |

I am greatly indebted to the authorities of the John Innes Horticultural Institution for allowing a large number of plants to be grown there. The assistance thus extended to me has enabled me largely to increase the scope of my experiments upon *Primula sinensis.*

# *The Action of Animal Extracts on Milk Secretion.*

By E. A. Schäfer, F.R.S., and K. Mackenzie, M.B., Ch.B.

(Received March 7,—Read March 9, 1911.)

(From the Department of Physiology, University of Edinburgh.)

Since the secretion of milk is known to proceed with the same regularity whether the nerves to the mammary glands are cut or intact*, it seems probable that it is provoked by other than nervous stimuli. It cannot, indeed, be contested that the secretion is influenced through the nervous system, but this may be indirect, if the formation and outpouring of the secretion can be shown to be produced by chemical agents (hormones) circulating in the blood, such as have been found to excite secretion in the pancreas,† which is stimulated to active secretion by a material obtained from the mucous membrane of the duodenum, and in the kidney,‡ which is stimulated by a material yielded by the posterior or infundibular portion of the pituitary body.

We have investigated the action of a large number of animal extracts upon the flow of milk from the mammary glands of lactating animals, chiefly cats, but including some dogs. The extracts, which were made with Ringer's solution, and were in most cases previously boiled, were injected slowly and in small amount (not more than 5 c.c. at a time) into a superficial vein, and the flow of milk, if any, was recorded by one of two methods, or by both methods simultaneously. The simpler method consists in recording the rate of exudation of milk from a small and superficial cut into one of the mammary glands (exudation method). The other method consists in recording the flow of milk led from a canula tied into a cut nipple (nipple method); in either case the milk is allowed to drop upon an electric recorder, and the drops are marked by an electromagnetic signal upon the paper of a kymograph. On this paper are also recorded at the same time in some of our experiments the blood-pressure, the volume of the kidney, and the rate of excretion of urine. The animals were anæsthetised either with chloroform alone or with chloroform followed by chloral, the latter being administered either intravenously or subcutaneously; after the complete effect of the chloral is established, the chloroform administration is stopped.

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* Eckhard, 'Beiträge zur Anat. u. Physiol.,' 1855 and 1897.

† Bayliss and Starling, 'Journ. Physiol.,' 1902, vol. 28, and 1903, vol. 29.

‡ Schäfer and Herring, 'Phil. Trans.,' 1906, B, vol. 199.

Until quite recently nothing had been ascertained regarding the influence of animal extracts upon milk secretion. But in a type-written notice inserted into a pamphlet on "Internal Secretions," by Dr. Isaac Ott, of Philadelphia, and dated October 28, 1910, the brief statement is made that "infundibulin is a rapid and powerful galactagogue."* This pamphlet came into our hands on November 20, and the statement in question furnished the starting point of our investigations.

The animal extracts which we have investigated are numerous, and include not only extracts of both parts of the pituitary body, but also extracts of placenta,† uterus in process of involution, mammary gland, duodenum, liver, spleen,‡ kidney, thyroid, ovary, testicle, thymus, and suprarenal capsules. In the ovary we investigated separately the ovarian substance proper and the substance of the corpora lutea. And in view of the growth of mammary gland substance which was found by Miss Lane-Claypon and Prof. Starling§ to be produced in the (virgin) rabbit by hypodermic injections of extract of rabbit-fœtuses, we also tried the effect on the secretion of the mammary gland (of the cat) of intravenous injection of extract of cat-fœtuses, with, however, a negative result. The most constant positive results which we have obtained have been those resulting from extracts of the posterior lobe of the pituitary body (of the ox) and of corpus luteum (of the sheep). Of these two materials that contained in the posterior lobe of the pituitary body is the more active. The accompanying curve (fig. 1) exhibits the effect produced by intravenous injection of a small amount of this extract. Prior to the injection no milk was passing from the gland; indeed, in the absence of a special stimulus the secretion almost always remains in abeyance. But within 20 seconds of the injection, drops of milk began to fall fast from the tube, the end of which was little, if at all, below the level of the gland with which it was connected, so that the flow was not assisted by suction, but must have been the result of the *vis a tergo* of the secretion. The effect

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* "Infundibulin" appears to be a proprietary article and is described by Ott (p. 57) as "a 20-per cent. extract of the pituitary." But probably, as the name implies, the infundibular part of the gland is alone used in its preparation.

† Lederer and Pribram ("Report of Internat. Physiol. Congress, Vienna," 'Zentralbl. f. Physiol.,' 1910, vol. 24, p. 817) state that they have obtained increase of milk secretion in the goat as the result of intravenous injection of unboiled extract of placenta, but our results with this extract have so far been negative (in the cat and dog).

‡ In investigating the action of spleen extracts we have incidentally found that such extracts may produce marked diuresis, without either rise of blood-pressure (in point of fact the blood-pressure falls) or increase of kidney volume. The diuresis must therefore be caused by a direct stimulating action upon the secreting cells of the kidney.

§ 'Roy. Soc. Proc.,' B, 1906, vol. 77.

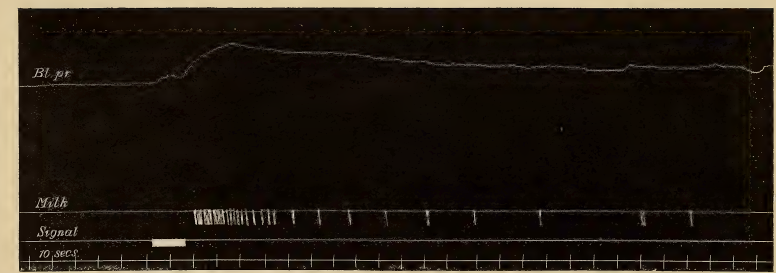

Fig. 1.—Graphic record of the milk secretion which was produced in a lactating cat as the result of intravenous injection of 4 c.c. Ringer's solution containing 1·5 c.c. of pituitrin solution (Parke, Davis and Co.) prepared from extract of posterior lobe of ox-pituitary. The uppermost line records the blood-pressure ; the second the dropping of milk from a tube connected with a cut nipple ; the drops fell at first so fast as to run together in the record ; the third line marks the signal of injection ; the fourth line time-intervals of 10 secs.

passed off after three or four minutes, during which time 30 to 40 large drops of milk were recorded. This, it must be remembered, was from two of the mammary glands only (usually two were led off to the one drop-recorder), representing about one-fifth of the whole glandular mass. But it lasts only a short time, in which respect it differs from the effect which the same extract produces upon the urinary secretion, the rate of which may be increased during many minutes after the injection, and even to some degree for a prolonged period. In the case of the urinary secretion, a second or "repeat" dose of posterior-lobe extract, given within a period of half an hour or less, causes a renewed increase of urine secretion, although this may be now unaccompanied by a repetition of the rise of blood-pressure which accompanies the first dose.* But the effect of a repeat dose upon the secretion of the mammary gland is much less than that produced by the first dose, and in some cases fails to be recorded by the "nipple method," although it can be sometimes observed by the "exudation method"; and such repetition produces a smaller result than the previous one.

We find that the galactagogue substance of the pituitary body is not present in the pars anterior, but only in the pars intermedia and pars posterior of the gland. It is not yielded to absolute alcohol, although a very small amount of water in the alcohol used will suffice to extract it. It is not destroyed by contact with absolute alcohol (at least within a reasonable period), nor by repeated boiling, nor by prolonged keeping in the dry state (we have obtained marked effect from dry posterior-lobe substance which has been kept some years in a stoppered bottle). The galactagogue action runs parallel in time with the action of the extract upon the systemic blood-vessels, which are contracted by posterior-lobe extracts.† It is probable, however, that, as in the case of the kidney, the blood-vessels of the mammary gland do not share in the general constriction which this extract produces. But we have not yet succeeded in definitely determining by a plethysmographic method whether the vessels of the gland dilate during the increased secretion, although to judge from the appearance of the cut gland this would seem to be the case. This is a point which must be the subject of further investigation.

Another extract which in our hands has yielded a definite positive result is extract of fresh corpus luteum, prepared with Ringer's solution. The effect is quite distinct but less decided than with extract of posterior lobe of pituitary: for, instead of some 30 to 40 drops, not more than 5 drops of milk were yielded by the nipple method after injection of 5 c.c. of a corpus luteum extract made up in the proportion of 1 part of the fresh tissue to 10 parts of Ringer's solution. One such result is shown in fig. 2, A. The active substance of the corpus luteum is probably not the same as that obtained from the pituitary, for its galactagogue action is unaccompanied by the same general rise of blood-pressure; indeed, there is usually a fall of a more or less decided character. As with the pituitary material, the galactagogue substance of the corpus luteum is not yielded to absolute alcohol, nor destroyed by contact with alcohol (at least for a short time) nor by repeated boiling. And, as with pituitary extract, a repeat dose is usually less effective as compared with the first dose; the amount of milk formed under its influence is often insufficient to cause the secretion to flow from the nipple, although secretion may be apparent when the exudation method is adopted. A second effect is, however, distinct in fig. 2, B.

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* Schäfer and Herring, *op. cit.*

† Oliver and Schäfer, 'Journ. Physiol.,' vol. 18, 1895.

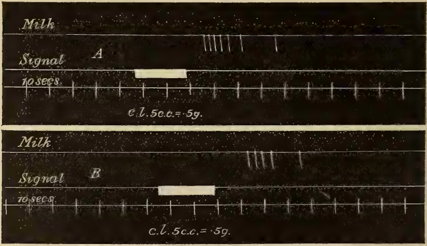

Fig. 2.—Records of milk-flow from a nipple of a lactating cat as the result of the intravenous injection of 5 c.c. of Ringer's solution extract of corpus luteum from sheep's ovary. Two such injections were given. In the record of the first (A) six drops are shown; in that of the second (B) five drops. Signal and time tracings as in fig. 1.

In order to produce the galactagogue effect, it is not necessary to employ a lactating animal. In one instance we obtained a free flow of fluid—of serous appearance—from the incised mamma of a cat, apparently virgin, and not fully grown. This is illustrated in the tracing which is shown in fig. 3, which is from a small cat, weighing $2\frac{1}{2}$ kilogrammes and rather more than three parts grown. The mammary glands were very small and little developed, and confined to the neighbourhood of the nipples. The fluid which exuded from the incised gland was led by a wet cotton wick over a drop-recorder and the drops were marked in the usual way by electromagnetic signal. Prior to the injection (of the corpus luteum extract) no flow was registered, but within less than a minute of the injection the flow began and was, in fact, at first so rapid that two or three drops fell at the side and escaped registration. The galactagogue action in this case lasted about ten minutes. A second dose proved ineffective.

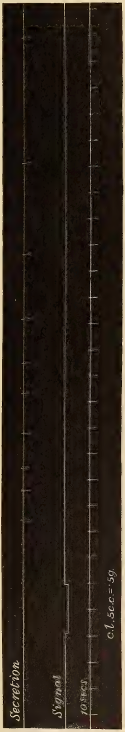

FIG. 3.—Record of flow of secretion from the mammary gland of a non-lactating and apparently virgin cat as the result of intravenous injection of 5 c.c. of Ringer's solution extract of corpus luteum from sheep's ovary (exudation method). The uppermost line shows the drops of secretion; signal and time tracings as in the preceding figures.

We have further investigated the action of a number of drugs which from their influence on other glands might have been expected to influence the secretion of the mammary gland; but with negative results. Amongst these may be mentioned pilocarpine, eserine, and nicotine. A dose of pilocarpine capable of producing intense salivation and lacrimation has no perceptible influence on milk secretion. Secretine also gives a negative result. Nor have we so far succeeded in obtaining any positive result from the electrical excitation of the nerves to the glands. Our experiments in this direction have not been sufficiently numerous for us to state definitely that no effect is under any circumstances so obtainable, but hitherto neither by reflex nor by direct stimulation have we been able to cause a flow of milk from the nipple. The work in this and in some other directions is, however, still in progress, and the results will be given in a later communication.

Within the last few days, and since the completion of our joint work upon this subject,* there has come into our hands the number of the 'Proceedings of the Society of Experimental Biology of New York,' which describes the communications made to that Society at its meeting on December 21, 1910. Amongst these communications are two by Drs. Isaac Ott and J. C. Scott, which deal with the galactagogue action not only of "infundibulin" but also of other animal extracts. These authors have used the goat as the subject of their experiments, determining the amount of milk which could be drawn from the udder by an aspirator before and after the injection of various extracts into a vein of the ear. They record a very striking action of infundibulin, the amount of secretion being increased in one experiment as the result of a single injection from 5 drops to 400 drops of milk in periods of five minutes; they have also obtained a galactagogue action from extracts of corpus luteum, thymus and pineal gland. Although we have not been able to determine this action in the case of the two last mentioned glands, our experiments upon the pituitary and corpus luteum have yielded results, in the cat and dog, similar to those obtained by Drs. Ott and Scott in the goat, and we shall await with interest the publication of the details of their experiments. In any case the credit of the discovery of hormones which influence milk secretion belongs to them, and our results, although arrived at on other animals and by a somewhat different method, are in the main confirmatory of those which the American authors have established, at least for the early period of lactation.

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* The experiments are being continued by Dr. Mackenzie alone.

[*Note added March* 31, 1911.—Since this paper was read, Dr. Mackenzie has found that extracts both of involuting uterine mucous membrane and of mammary gland itself are markedly galactagogue, and that with regard to the action of pituitary extract, the source of this extract appears to make no difference to its activity; the extract of the bird's pituitary being quite as active in promoting the mammary secretion as that of the mammalian pituitary itself. He has also determined that atropine does not interfere with the action of any of these galactagogues.]

# *Inbreeding in a Stable Simple Mendelian Population with Special Reference to Cousin Marriage.*

By S. M. Jacob, I.C.S., Biometric Laboratory, University College, London.

(Communicated by Prof. Karl Pearson, F.R.S. Received March 18,—Read May 18, 1911.)

CONTENTS.

PAGE.
1. Introductory ........ 23
2. Brother-Sister Marriages........ 24
3. First Cousin Marriages ........ 26
4. General Sibling Families ........ 29
5. *n*th Cousin Marriages ........ 31
6. Second Cousin Marriages in Detail ........ 33
7. Numerical Proportions of Constituents for First Cousin Marriages ... 34
8. Percentage of Allogenic Offspring which arise from First Cousin Marriages ........ 35
9. Relative Rate of Production of Allogenic Constituents by First Cousins and Non-related Pairs ........ 38
10. Conclusions and Limitations ........ 40

1. The Mendelian theory of the segregation of unit characters, though it is far, as yet, from being completely demonstrated, offers a simple explanation of some striking features of inheritance. In particular, Mr. E. C. Snow has recently shown* that the gametic correlations for collaterals deducible from the Mendelian hypothesis are in close agreement with the actually observed somatic correlations for man and certain other animals; or, in other words, that a Mendelian theory of segregation without dominance gives values for collateral resemblance not greatly differing from those found from observation.†

It seems, therefore, possible that the same theory will throw some light on the problem of inbreeding, or, at any rate, will indicate to what points, on which precise data are at present lacking, statistical enquiry should be directed. Without these data the Mendelian theory cannot be corroborated or negatived by the methods of the present paper. So far as they go, however, the statistics at present obtainable with regard to consanguinity in the parentage of albinos and deaf mutes are in approximate agreement with the calculated results, although the accuracy of the figures is too uncertain for the application of anything more than a rough criterion.

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* 'Roy. Soc. Proc.,' B, vol. 83, 1910.

† Prof. Pearson had previously shown that this result is true for the ancestral relationships, 'Roy. Soc. Proc.,' B, vol. 81, 1909.

The paper also gives a very simple operational notation by which the sibling families in any generation can be at once written down. From this result the offspring of the sibling group in any generation, when these mate endogamously, can be immediately calculated.

As Mendelism, whether it be finally accepted or rejected, is likely to be a very much debated subject for some time to come, this condensed notation may serve a useful purpose in minimising algebraical labour. Miss Elderton and Prof. Karl Pearson have taken a special case of Mendelian inbreeding,* in which one of the common grandparents of the inbreeding first cousins is a pure recessive, although the occurrence of this pure recessive constituent is assumed to be excessively rare in the population at large. If the recessive constituent is a harmful one this makes first cousin marriages appear in a very unfavourable light. As a matter of fact it will be shown that where the recessive element is of very infrequent occurrence inbreeding greatly increases the relative chances of its appearance in the offspring of consanguineous matings as compared to its frequency in the offspring of unrelated pairs; but at the same time the absolute frequency becomes smaller and smaller in both cases with increasing rarity of the pure recessive in the general population.

In the present paper the ancestry of the inbreeding sibling group is always supposed to be a representative sample of the general stable population.

The plan adopted will be, first of all, to obtain the offspring of brother-sister and first cousin marriages in the direct way, and then to use the operational notation to obtain the offspring of intermarrying $n$th cousins. Some numerical results have been calculated, and a comparison made with the available statistics.

The notation used will be the ordinary, though verbally somewhat inconvenient one in which (AA), (A$a$), ($aa$), represent the protogenic, hybrid, and allogenic constituents formed by a single allelomorphic pair.

Where only a single pair of allelomorphs is dealt with, the only possible stable form of Mendelian community, in which there is no preferential mating, selective death-rate, or differential fertility, is

$$p^2\,(\mathrm{AA}) + 2pq\,(\mathrm{A}a) + q^2\,(aa),$$

where the coefficients give the frequency of the elements which they precede.

2. In order to obtain the offspring of brother-sister marriages we must take each of the families shown in Table I on p. 41 of Mr. Snow's paper, and, assuming that these first siblings consist of males and females in equal

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* 'Eugenics Laboratory Memoirs,' IV, "On the Measure of the Resemblance of First Cousins." Dulau and Co., 1907.

numbers, mate them in pairs, and multiply the offspring by the frequency of the particular type of family.

The total number of children in each first sibling family is taken as $16s$, where $s$ does not vary from family to family. In practice, of course, as every family does not consist of a fixed number of children with the brothers and sisters equally numerous, this assumption would appear to be rather a large one. It will be necessary to consider later how far the constancy of $s$, and its equal partition into male and female, are hypotheses likely to give reasonable approximations.

Certainly the complexity of the analysis would be very greatly increased if every possible size of family, with every possible distribution of the sexes, were considered, and probably no very great modification of the result is to be anticipated.

Let the offspring of each brother-sister marriage be $4t$, where $t$ is also constant, but may differ from $4s$.

In the first family there are $8s$ brothers and $8s$ sisters. There will be $8s$ matings of brother and sister, and the progeny will be $8st\,4\,(\text{AA})$.

In the second type of family there are $4s$ brothers (AA), and $4s$ brothers (A$a$), and a like distribution of sisters. The progeny will be

$$2st\,\{9\,(\text{AA}) + 6\,(\text{A}a) + (aa)\}.$$

In the third type there are $8s\,(\text{A}a)$ brothers, and the progeny is

$$8st\,\{(\text{AA}) + 2\,(\text{A}a) + (aa)\}.$$

In the fourth type there are $2s\,(\text{AA}) + 4s\,(\text{A}a) + 2s\,(aa)$ brothers, and the progeny is $8st\,\{(\text{AA}) + 2\,(\text{A}a) + (aa)\}$.

In the fifth type there are $4s\,(\text{A}a) + 4s\,(aa)$ brothers, and the progeny is

$$2st\,\{(\text{AA}) + 6\,(\text{A}a) + 9\,(aa)\}.$$

In the sixth type there are $8s\,(aa)$ brothers, and the progeny is $8st\,4\,(aa)$.

The frequency of these types is $p^4$, $4p^3q$, $2p^2q^2$, $4p^2q^2$, $4pq^3$, $q^4$.

Multiplying the offspring by these frequencies and adding, we find the total offspring of all types of brother-sister marriages is (omitting the factor $2st$):—

$$\begin{aligned}
&16p^4(\text{AA})\\
&+36p^3q(\text{AA})+24p^3q(\text{A}a)+4p^3q(aa)\\
&+\ 8p^2q^2(\text{AA})+16p^2q^2(\text{A}a)+8p^2q^2(aa)\\
&+16p^2q^2(\text{AA})+32p^2q^2(\text{A}a)+16p^2q^2(aa)\\
&+\ 4pq^3(\text{AA})+24pq^3(\text{A}a)+36pq^3(aa)\\
&\qquad\qquad\qquad\qquad\qquad\quad+16q^4(aa).
\end{aligned}$$

Dividing by 4 (the factor $8st$ is thus omitted), and collecting like terms, the result is

$$(p+q)^2[p(4p+q)(\mathrm{AA})+6pq(\mathrm{A}a)+q(p+4q)(aa)].$$

Thus the progeny of brother-sister marriages is $8\,st\,(p+q)^2$ multiplied by

$$p(4p+q)(\mathrm{AA})+6pq(\mathrm{A}a)+q(p+4q)(aa). \qquad (1)$$

This last expression gives the relative frequency of the protogenic, hybrid, and allogenic constituents in the offspring.

The percentage of pure recessives in the offspring of brother-sister marriages is $\dfrac{100\,q\,(p+4\,q)}{4\,(p+q)^2}$, as against a percentage of $\dfrac{100\,q^2}{(p+q)^2}$ in the offspring of unrelated pairs.

The relative frequency is the ratio of $1+\dfrac{p}{4\,q}$ to unity. This increases indefinitely with the ratio $p$ to $q$, that is with the greater rarity of the allogenic element in the general non-inbreeding population. This result will be seen to hold for the offspring of inbreeding couples, however distantly related. For this reason the discussion of this point will be postponed for fuller consideration when cousin marriages are dealt with.

The absolute frequency of the allogenic constituent in the offspring of brother-sister marriages is $\dfrac{1+p/4\,q}{(1+p/q)^2}$, which approaches zero when $p/q$ is very large.

In the case given by Miss Elderton and Prof. Pearson this ratio is $1/4$. For this value of the ratio, $p/q = 1{\cdot}3$ approximately, and the proportion of the allogenic constituent in the non-inbreeding population becomes $1/5{\cdot}29$. Thus, for an evil which is recessive and obeys the Mendelian formula, its appearance in 25 per cent. of the offspring of brother-sister marriages must be compared with 18·9 per cent. of patent evil in the offspring of non-inbreeding couples.

3. We will now turn to first cousin marriages. Mr. Snow's Table IV can be shortly written out by collecting like terms in each sibling family, and the same process as that used for obtaining the offspring of brother-sister marriages can be readily applied. The fertility of the first sibling generation when mated to similar first sibling families is taken to be $4t$. It is not necessary to give here the reduced table.

Each constituent in Mr. Snow's Table IV denotes that there are $16s$ families, each with $4t$ members, and these members are brothers and sisters to members of the same family, and first cousins to members of other families in the group. Thus in all there are in each group $64st$ individuals who are either brothers and sisters or first cousins.

As before we assume an equi-partition of the sexes, that is there are to be $32\,st$ males and $32\,st$ females in each group. Not only are males and females to be equally numerous in the group as a whole, but also in each family is the number of protogenic males to be equal to the number of protogenic females, and so on for each type of constitution.

If we number the groups in Mr. Snow's table from 1 to 36, from left to right taking the rows in turn, we have 15 different types of second sibling family. Then, mating male and female, and putting the fertility of each mating as $4m$, the offspring of inbreeding second siblings is

$$\begin{aligned}&(\mathrm{AA})\,[8p^8+49p^7q+54p^6q^2+25p^5q^3+4p^4q^4+72p^2q^6+150p^5q^3\\&\qquad+64p^4q^4+9p^3q^5+72p^4q^4+54p^3q^5+8p^2q^6+pq^7+6p^2q^6]\\&+(\mathrm{A}a)\,[14p^7q+36p^6q^2+30p^5q^3+8p^4q^4+48p^6q^2+180p^5q^3\\&\qquad+128p^4q^4+30p^3q^5+144p^4q^4+180p^3q^5+48p^2q^6+14pq^7+36p^2q^6]\\&+(aa)\,[\text{the expression for the coefficient of (AA) with } p \text{ and } q \text{ interchanged}].\end{aligned}$$

When the terms are collected, this reduces to

$$(p+q)^6\,[(8p+q)\,p\,(\mathrm{AA})+14pq\,(\mathrm{A}a)+(p+8q)\,q\,(aa)]. \tag{2}$$

This last expression, multiplied by $16\,stm$, gives the distribution of children of all second siblings. Now we have mated together at random all the second siblings in each group of families, so that both first cousin marriages and brother-sister marriages have been included. To obtain the offspring of first cousin marriages only we must subtract from the above result the offspring of brother-sister marriages in the second generation. These can be found from the expression for the children of brother-sister marriages in the first generation by changing $16\,s$ into $4t$, $t$ into $m$ multiplying by $16\,s\,(p+q)^4$, and dividing by $16\,s$.

The first three changes are clear. The final division by $16\,s$ is necessary, because, without it, we should obtain the offspring of second siblings, on the supposition that all these siblings contracted brother-sister marriages; whereas, if, as has been done above, the second siblings mate at random each in their own group of families, there will be $16\,s$ first cousin marriages to one brother-sister marriage, there being $16\,s$ families in each group.

Hence the children of brother-sister marriages in the second generation are given by the expression (1) multiplied by $2\,mt\,(p+q)^6$. Subtracting this from the expression for the offspring of all second siblings, we get, omitting the factor $2\,mt\,(p+q)^6$,

$$\begin{aligned}&8s\,[(8p+q)\,p\,(\mathrm{AA})+14pq\,(\mathrm{A}a)+(p+8q)\,q\,(aa)]\\&\qquad-[(4p+q)\,p\,(\mathrm{AA})+6pq\,(\mathrm{A}a)+(p+4q)\,q\,(aa)]\\&=[(64s-4)p+(8s-1)\,q]\,p\,(\mathrm{AA})+[112s-6]\,pq\,(\mathrm{A}a)\\&\qquad+[(8s-1)p+(64s-4)\,q]\,q\,(aa).\end{aligned} \tag{3}$$

The proportion of the allogenic constituent in the progeny of first cousin marriages is

$$\frac{[(8s-1)p+4(16s-1)q]q}{4(16s-1)(p+q)^2} = \frac{1+\dfrac{8s-1}{4(16s-1)}\dfrac{p}{q}}{\left(1+\dfrac{p}{q}\right)^2}. \tag{4}$$

The excess of the recessive constituent over the amount produced by the non-inbreeding population is

$$\frac{(8s-1)\dfrac{p}{q}}{4(16s-1)\left(1+\dfrac{p}{q}\right)^2}. \tag{5}$$

The ratio of the two rates of production of allogenic element by first cousin and non-consanguineous marriage respectively, is

$$1+\frac{8s-1}{4(16s-1)}\frac{p}{q}. \tag{6}$$

The question now arises as to what value should be given to $s$. Theoretically, even in man, the possible gametes of both male and female are very large in number, and the resulting zygotes would also be very numerous. It seems not unreasonable then to take the actual population, in which each family is limited by a variety of causes, of which no account can possibly be taken, as a random sample of the population which would arise, if every possible zygote were formed and developed. In this case we must put $s$ practically infinite. In doing this we do not make each individual family infinite, but we assume that existing families are a random sample of what would occur, if every possible gamete of both parents survived to form a zygote. This, of course, would not be legitimate if applied to the individual family, but it seems reasonable as a means of predicting the constitution of the whole population. To assume that each family has a variable number—as a rule too few to give the possible Mendelian variations—would lead to an immense algebraical extension of the work, and it is hard to see that it could lead to results differing from the supposition that the actual population is a random sample of the theoretically possible population, as an indication of the variation produced in the results by limiting fertility. I have put $16s$ equal also to the average size of the family met with in practice. The chief objection to this is that it obviously gives fractional frequencies, within the individual family, to some of the Mendelian possibilities.

In the present case, to make $s$ infinite means that the expression (2) alone gives the offspring of first cousin marriages, whereas it actually includes the offspring of the brother-sister marriages in the second sibling generation.* The inclusion of these marriages has the effect of increasing the proportion of protogenic and allogenic element at the expense of the hybrid constituent.

Seeing, then, that the proportion of allogenic element in the offspring of first cousins increases with the fertility of the first generation, where the fertility does not vary from family to family, it would appear not unlikely that where the fertility is made variable from mating to mating, the proportion of allogenic elements will not be greater than the value determined by putting the fertility constant and equal to the maximum value recorded in the observations. Further, it would appear that the true value must be greater than that given by the least recorded fertility.

It is true that the gametic correlations of Mendelian collaterals, which, by putting $s$ infinite, agree closely with statistical results for somatic characters, become too small when we put $16s = 4$, say. We have, in fact, the values $\frac{1}{3}$, $\frac{1}{6}$, $\frac{1}{6}$, for the fraternal, avuncular, and first cousin gametic correlations respectively. However, until Mendelism is more firmly established, this *ex-post-facto* justification, though of some weight, cannot be regarded as decisive.

In the numerical calculations that follow, the results have been given both for $s$ infinite and for $16s = 4$.

Before considering, however, the numerical consequences of the results obtained for first cousin marriages, the general expression for the progeny arising from the intermarriage of any grade of cousin will be investigated.

4. If we take two populations of the forms $\alpha_1(\mathrm{AA})+\beta_1(\mathrm{A}a)+\gamma_1(aa)$ and $\alpha_2(\mathrm{AA})+\beta_2(\mathrm{A}a)+\gamma_2(aa)$, where $\alpha_1+\beta_1+\gamma_1 = \alpha_2+\beta_2+\gamma_2$, and mate them at random in every possible way, the offspring of each mating numbering $4m$, the result is easily shown to be

$$[(2\alpha_1+\beta_1)\,\mathrm{A}+(\beta_1+2\gamma_1)\,a]\,[(2\alpha_2+\beta_2)\,\mathrm{A}+(\beta_2+2\gamma_2)\,a],$$

where, after algebraical multiplication, (AA) is written for $\mathrm{A}^2$, $(\mathrm{A}a)$ for $\mathrm{A}a$, and $(aa)$ for $a^2$. Hence the rule for finding the offspring of any two populations is easily seen. We simply write down for each population the number of each kind of allelomorph and add. We thus obtain two factors which, algebraically multiplied, give the required resultant offspring, provided $\mathrm{A}^2$, $\mathrm{A}a$, $a^2$, are interpreted as noted above. Thus, in the first of the two populations given above, the numbers of dominant and recessive allelomorphs are respectively $2\alpha_1+\beta_1$ and $\beta_1+2\gamma_1$, and these, multiplied by A and $a$ respectively, give the first of the required factors.

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* This of course indicates that brother-sister marriages, when all matings are random, would only form an indefinitely small fraction of cousin marriages.

Disregarding for the present the absolute fertilities in each generation, we see that the families of brethren or first siblings can be written in the symbolic form

$$[p^2(2\mathrm{A})+2pq(\mathrm{A}+a)+q^2(2a)]^2,$$

where the ordinary algebraical operations are first carried out, and then interpreted in the way noted.

Similarly, it is easily verified that the second sibling groups of families can be written symbolically in the form

$$[p^4(4\mathrm{A})+4p^3q(3\mathrm{A}+a)+6p^2q^2(2\mathrm{A}+2a)+4pq^3(\mathrm{A}+3a)+q^4(4a)]^2.$$

Let us assume that this result is generally true. We would then have the $n$th sibling groups given by

$$[p^{2^n}(2^n\mathrm{A})+{}_{2^n}\mathrm{C}_1p^{2^n-1}q\{2^n-1)\mathrm{A}+a\}+{}_{2^n}\mathrm{C}_2p^{2^n-2}q^2\{(2^n-2)\mathrm{A}+2a\}+\dots$$
$$+{}_{2^n}\mathrm{C}_lp^{2^n-l}q^l\{(2^n-l)\mathrm{A}+la\}+\dots+q^{2^n}(2^na)]^2. \quad (7)$$

We will prove that if this result holds for the $n$th siblings, it will be true for the $(n+1)$th siblings, and thus, inductively, that it will be universally true. For brevity write $2^n = w$.

First of all the types of families in the $(n+1)$th generation will be obtained, and then their frequencies.

The general type of family in the $n$th generation is given by

$$[(w-l_1)\mathrm{A}+l_1a][(w-l_2)\mathrm{A}+l_2a],$$

where $l_1$ and $l_2$ have any values from 0 to $w$.

This gives a group of type

$$(w-l_1)(w-l_2)(\mathrm{AA})+\{l_1(w-l_2)+l_2(w-l_1)\}(\mathrm{A}a)+l_1l_2(aa).$$

This family group written in operational form is—

$$\{2(w-l_1)(w-l_2)+l_1(w-l_2)+l_2(w-l_1)\}\mathrm{A}$$
$$+\{l_1(w-l_2)+l_2(w-l_1)+2l_1l_2\}a$$
$$=\{2(w-l_1)w-(w-l_2)l_1+(w-l_1)l_2\}\mathrm{A}+w(l_1+l_2)a$$
$$=w(2w-l_1-l_2)\mathrm{A}+w(l_1+l_2)a,$$

where $l_1+l_2$ can take any value from 0 to $2w$.

Thus the $(n+1)$th sibling group will be formed of types of family produced by two operators of the form

$$(2w-r)\mathrm{A}+ra,$$

where $r$ has any value from 0 to $2w$.

Thus the types of family groups in the $(n+1)$th sibling generation are given by

$$\left\{\sum_{=0}^{r=2w}[(2w-r)\mathrm{A}+ra]\right\}^2$$

We shall now determine the frequencies. From (7) we see that the frequency of the general type of family group in the $n$th sibling generation is given by the product

$$ {}_{w}C_{l_1} p^{w-l_1} q^{l_1} \times {}_{w}C_{l_2} p^{w-l_2} q^{l_2}, $$

where $l_1$ and $l_2$ can take any value from 0 to $w$.

Now, whenever $l_1 + l_2 = r$, we get an operator of the form $(2w-r)\,\mathrm{A} + ra$ Hence the frequency of this type of operator is

$$ \Sigma\, {}_{w}C_{l_1} p^{w-l_1} q^{l_1} \times {}_{w}C_{l_2} p^{w-l_2} q^{l_2} \text{ (where } l_1 + l_2 = r). $$

$$ = \sum_{l_1=0}^{l_1=r} {}_{w}C_{l_1} \cdot {}_{w}C_{r-l_1} p^{2w-r} q^{r}. $$

Now take the identity

$$ (1+x)^{w}(1+x)^{w} = (1+x)^{2w}, $$

and equate the coefficients of $x^r$ on both sides.

We have at once
$$ \sum_{l_1=0}^{l_1=r} {}_{w}C_{l_1} \cdot {}_{w}C_{r-l_1} = {}_{2w}C_{r}. $$

Hence the frequency of the operator of type $(2w-r)\,\mathrm{A} + ra$ is ${}_{2w}C_r p^{2w-r} q^r$ This is the operator which operating on itself produces the $(n+1)$th sibling families. Hence the $(n+1)$th generation is given symbolically by

$$ \left[\sum_{r=0}^{2w} {}_{2w}C_r p^{2w-r} q^r \left\{(2w-r)\,\mathrm{A} + ra\right\}\right]\Bigg]^2 $$

$$ = \left[\sum_{r=0}^{r=2^{n+1}} \left[{}_{2^{n+1}}C_r p^{2^{n+1}-r} q^r \left\{(2^{n+1}-r)\,\mathrm{A} + ra\right\}\right]\right]^2. \tag{8} $$

Thus it is shown that if the result (7) be true for the $n$th sibling generation, it is true for the $(n+1)$th. But it has been verified for the first and second sibling generation. Hence it is universally true.

5. To obtain the offspring when the $n$th generation of siblings inbreeds, we take each of the families in (7) and mate each type with itself. The result will be given by taking in (8) only the square terms, and omitting the cross-products, except that the frequencies are not to be squared, as we divide each group into an equal number of males and females, as has been done throughout.

We have then, neglecting absolute frequency, the offspring of inbreeding $n$th sibling groups given by

$$ \sum_{r=0}^{r=2w} \left[\left\{(2w-r)^2 (\mathrm{AA}) + 2r(2w-r)(\mathrm{A}a) + r^2 (aa)\right\} \times {}_{2w}C_r p^{2w-r} q^r\right]. $$

Consider first the coefficient of (AA). It is

$$ \sum_{r=0}^{r=2w} (2w-r)^2\, {}_{2w}C_r p^{2w-r} q^r. $$

Now let us evaluate

$$2w \times {}_{2w-2}\mathrm{C}_r + {}_{2w-2}\mathrm{C}_{r-1} = \frac{2w(2w-2)!}{(2w-2-r)!\,r!} + \frac{(2w-2)!}{(2w-1-r)!\,(r-1)!}$$

$$= \frac{(2w-2)!\,\{2w(2w-r-1)+r\}}{(2w-r-1)!\,r!}$$

$$= \frac{(2w-1)!\,(2w-r)}{(2w-r-1)!\,r!} = \frac{(2w-r)^2}{2w}\,{}_{2w}\mathrm{C}_r.$$

Thus

$$\sum_{r=0}^{r=2w} (2w-r)^2\,{}_{2w}\mathrm{C}_r p^{2w-r} q^r$$

$$= \sum_{r=0}^{r=2w-1} 2w\left[2w \times {}_{2w-2}\mathrm{C}_r + {}_{2w-2}\mathrm{C}_{r-1}\right] p^{2w-r} q^r$$

$$= 2w(2wp+q)\,p \sum_{r=1}^{r=2w-1} {}_{2w-2}\mathrm{C}_r p^{2w-r-1} q^{r-1}$$

$$= 2w(2wp+q)\,p\,(p+q)^{2w-2}.$$

Take next the coefficient of (A$a$). It is

$$\sum_{r=1}^{r=2w-1} 2r(2w-r)\,{}_{2w}\mathrm{C}_r p^{2w-r} q^r$$

$$= \sum_{r=1}^{r=2w-1} \frac{2(2w)!}{(2w-r-1)!\,(r-1)!} p^{2w-r} q^r$$

$$= \sum_{r=0}^{r=2w-2} \frac{2\,(2w)\,(2w-1)\,(2w-2)!}{(2w-r)!\,r!} p^{2w-r-1} q^{r+1}$$

$$= 2w(4w-2)\,pq \sum_{r=0}^{r=2w-2} {}_{2w-2}\mathrm{C}_r p^{2w-r-2} q^r$$

$$= 2w\,(4w-2)\,pq\,(p+q)^{2w-2}.$$

The coefficient of ($aa$) is clearly found by interchanging $p$ and $q$ in the coefficient of (AA), and it is

$$2w\,(p+2wq)\,q\,(p+q)^{2w-2}.$$

Thus the distribution of the constituents in the offspring of inbreeding $n$th siblings is (after dividing by $2\,w\,(p+q)^{\,2w-2}$),

$$(2wp+q)\,p\,(\mathrm{AA})+(4w-2)\,pq\,(\mathrm{A}a)+(p+2wq)\,q\,(aa).$$

Hence the offspring of $n$th cousins is

$$(2^{n+2}p+q)\,p\,(\mathrm{AA})+(2^{n+3}-2)\,pq\,(\mathrm{A}a)+(p+2^{n+2}q)\,q\,(aa). \qquad (9)$$

This, of course, includes the offspring of brother-sister, first cousin, second cousin, up to $(n-1)$th cousin marriages.

Thus the proportion of the allogenic element determined by (9) is $\frac{1}{(1+p/q)^2}+\frac{1}{2^{n+2}}\frac{p/q}{(1+p/q)^2}$, as against $\frac{1}{(1+p/q)^2}$ in the non-inbreeding population.

The ratio of the two quantities of allogenic constituent is

$$1+\frac{1}{2^{n+2}}\frac{p}{q},$$

which agrees for $s = \infty$ with the result already obtained for brother-sister and first cousin marriages.

6. We will now consider in more detail the offspring of intermarrying second cousins, introducing the necessary fertility factors and making the deductions for the inclusion of brother-sister and first cousin marriages in the result already obtained.

The distribution of all the offspring of second cousins, first cousins in the second generation, and of brother-sister marriages in the third generation is found by putting $n = 2$ in (9), and we have

$$p(16p+q)(\text{AA})+30pq(\text{A}a)+q(p+16q)(aa) \qquad (10)$$

The absolute frequency of each type must now be considered.

We started with $(p+q)^2$ individuals who mated in every possible way with $(p+q)^2$ other individuals, and had $16s$ offspring to each mating. Hence the first siblings number $16s(p+q)^4$.

The next fertility being $4t$, the second siblings number $64st(p+q)^8$.

The next fertility being $4m$, the third siblings number $256stm(p+q)^{16}$.

Let the fertility of third siblings inbreeding with each other be $4n$. Then the children of third siblings will number $1024stmn(p+q)^{16}$.

Now the number of individuals in (10) is $16(p+q)^2$. Hence (10) must be multiplied by $64stmn(p+q)^{14}$ to give the absolute frequencies.

Now in the expression (2) multiplied by $16stm$ we have the offspring of all first cousin and brother-sister marriages in the second generation. We must transfer this to obtain the offspring of first cousins in the second generation and of brother-sister marriages in the third generation, and subtract the result from the offspring of third siblings generally.

The result is obtained by changing $16s$ into $4t$, $t$ into $m$, $m$ into $n$ and multiplying by $16s(p+q)^8$, and finally dividing by $16s$, since the chance of a brother-sister and first cousin marriage in the random marriages of third siblings generally is $\frac{1}{16s}$ of the whole number of matings.

Hence the offspring of first cousin marriages in the second generation and of brother-sister marriages in the third generation is $64stmn(p+q)^8 \cdot \frac{1}{16s}$ multiplied by the expression (2). That is, we must subtract

$$4tmn(p+q)^{14}\left[(8p+q)p(\text{AA})+14pq(\text{A}a)+(p+8q)q(aa)\right]$$

from the expression for the offspring of inbreeding third siblings generally.

Omitting the common factor $4\,tmn\,(p+q)^{14}$, the result is

$$16s\,[p\,(16p+q)\,(\mathrm{AA})+30pq\,(\mathrm{A}a)+q\,(p+16q)\,(aa)] \\ -[p\,(8p+q)\,(\mathrm{AA})+14pq\,(\mathrm{A}a)+q\,(p+8q)\,(aa)].$$

Thus the offspring of second cousin marriages is given by

$$p\,[8p\,(32s-1)+(16s-1)\,q]\,(\mathrm{AA})+2pq\,(240s-7)\,(\mathrm{A}a) \\ +q\,[(16s-1)\,p+8q\,(32s-1)]\,(aa). \qquad (11)$$

The proportion of allogenic element is

$$\frac{1}{(1+p/q)^2}+\frac{(16s-1)\,p/q}{8\,(32s-1)\,(1+p/q)^2},$$

and the ratio of this to the amount of allogenic element in the non-inbreeding population is

$$1+\frac{16s-1}{8\,(32s-1)}\frac{p}{q}.$$

For $s=\infty$ this becomes $1+\frac{1}{16}\frac{p}{q}$, and for $16\,s=4$ it is $1+\frac{3}{56}\frac{p}{q}$, the corresponding values for first cousin marriage being $1+\frac{1}{8}\frac{p}{q}$ and $1+\frac{1}{12}\frac{p}{q}$.

7. We shall now proceed to consider the results obtained with particular reference to the marriage of first cousins. In the first place it will be necessary to examine how far the numerical proportions of pure dominant, hybrid, and pure recessive differ in the offspring of first cousins from the proportions in a non-inbreeding population.* For this purpose the ratio of $p$ to $q$ has been selected to give in the non-inbreeding population 25, 10, 5, 4, 3, 2, and 1 per cent. and the fractions 1/1,000, 1/10,000, 1/20,000, and 1/1,000,000 of pure recessive.

Table I gives the percentages of each type of constitution in the offspring of non-consanguineous and of consanguineous first cousin marriages.

This table illustrates the absolute decrease in the percentage of pure recessive in the offspring of consanguineous marriages, while it is seen that the ratio of the percentages of allogenic constitution steadily rises as the frequency of occurrence of the allogenic element diminishes.

If we have an evil which is a Mendelian recessive and is of common type, such as tuberculosis,† then first cousin marriage will not be much more likely to produce a defective offspring than any other kind of marriage.

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* By a "non-inbreeding population" in this paper is meant one in which inbreeding is not universal, *i.e.*, it corresponds to the general population resulting from random mating.

† It is not intended to imply that tuberculosis has been proved to depend on a simple Mendelian recessive factor.

Table I.

| | | (AA). | (A$a$). | ($aa$). |
|---|---|---|---|---|
| $p = q$ | In the non-inbreeding population | 25·00 | 50·00 | 25·00 |
| | Offspring of first cousins $16s = 4$ | 27·08 | 45·83 | 27·08 |
| | „ „ „ $s = \infty$ | 28·12 | 43·75 | 28·12 |
| $p = 2·162q$ | In the non-inbreeding population | 46·74 | 43·24 | 10·00 |
| | Offspring of first cousins $16s = 4$ | 48·54 | 39·64 | 11·81 |
| | „ „ „ $s = \infty$ | 49·45 | 37·84 | 12·70 |
| $p = 3·472q$ | In the non-inbreeding population | 60·27 | 34·27 | 5·00 |
| | Offspring of first cousins $16s = 4$ | 61·72 | 31·82 | 6·45 |
| | „ „ „ $s = \infty$ | 62·45 | 30·37 | 7·18 |
| $p = 4q$ | In the non-inbreeding population | 64·00 | 32·00 | 4·00 |
| | Offspring of first cousins $16s = 4$ | 65·33 | 29·33 | 5·33 |
| | „ „ „ $s = \infty$ | 66·00 | 28·00 | 6·00 |
| $p = 4·773q$ | In the non-inbreeding population | 68·35 | 28·64 | 3·00 |
| | Offspring of first cousins $16s = 4$ | 69·54 | 26·25 | 4·19 |
| | „ „ „ $s = \infty$ | 70·13 | 25·06 | 4·79 |
| $p = 6·071q$ | In the non-inbreeding population | 73·71 | 24·28 | 2·00 |
| | Offspring of first cousins $16s = 4$ | 74·73 | 22·26 | 3·01 |
| | „ „ „ $s = \infty$ | 75·23 | 21·24 | 3·52 |
| $p = 9q$ | In the non-inbreeding population | 81·00 | 18·00 | 1·00 |
| | Offspring of first cousins $16s = 4$ | 81·75 | 16·50 | 1·75 |
| | „ „ „ $s = \infty$ | 82·12 | 15·75 | 2·12 |
| $p = 30·62q$ | In the non-inbreeding population | 93·76 | 6·12 | 0·10 |
| | Offspring of first cousins $16s = 4$ | 94·01 | 5·61 | 0·36 |
| | „ „ „ $s = \infty$ | 94·14 | 5·36 | 0·48 |
| $p = 99q$ | In the non-inbreeding population | 98·01 | 1·98 | 0·01 |
| | Offspring of first cousins $16s = 4$ | 98·09 | 1·81 | 0·09 |
| | „ „ „ $s = \infty$ | 98·13 | 1·73 | 0·13 |
| $p = 140·4q$ | In the non-inbreeding population | 98·59 | 1·40 | 1/20,000 |
| | Offspring of first cousins $16s = 4$ | 98·63 | 1·29 | 12·7/20,000 |
| | „ „ „ $s = \infty$ | 98·68 | 1·23 | 18·8/20,000 |
| $p = 999q$ | In the non-inbreeding population | 99·80 | 0·20 | 1/1,000,000 |
| | Offspring of first cousins $16s = 4$ | 99·81 | 0·18 | 84/1,000,000 |
| | „ „ „ $s = \infty$ | 99·81 | 0·17 | 126/1,000,000 |

It is only for a rarely occurring evil which is recessive that consanguinity will probably have a marked effect.

8. Prof. Pearson has suggested to me that in order to compare the results obtained with statistical data, it is necessary to determine on the Mendelian hypothesis what percentage of pure recessive individuals, in a population in which there are both consanguineous and non-consanguineous marriages, is the offspring of related parents.

For the application of this test it is necessary to know what is the frequency with which cousin marriages occur. This frequency, however, is not known with any degree of certainty, various determinations which have been given ranging in value from as much as 1 to 8 per cent. of the total number of marriages. It is beyond the scope of this paper to consider the accuracy of these determinations, and they do not claim to be more than approximations.

So far as can be judged, however, a first cousin marriage rate of 5 per cent., such as may occur perhaps among the peerage, is not likely to be exceeded in any considerable group of the population, though it may apparently be as small as $1\frac{1}{4}$ or $1\frac{1}{3}$ per cent. for parts of the community of low social status. Accordingly, in what follows the determinations have been made on the basis of percentage rates of first cousin marriage from 1 to 5.

For the moment, only first cousin marriages are dealt with, all other marriages being assumed to be non-consanguineous.

Let $\lambda_1$ be the percentage of first cousin marriages. Let $e_1$ be the proportion of the offspring of first cousin marriages which has the allogenic constitution (*aa*). The value of $e_1$ is given by the preceding analysis.

Let $e$ and E be the proportions of allogenic constituent in the offspring of non-related couples, and in the population at large. As a rule, E will be the datum available from statistics.

Then, clearly, the total proportion of allogenic element in the whole population can be expressed in two ways, which together give rise to the equation

$$\lambda_1 e_1 + (100-\lambda_1)\, e = 100\text{E}.$$

Now, if we put $p/q = z$, we have

$$e_1 = \frac{1+g_1 z}{(1+z)^2}, \quad \text{and} \quad e = \frac{1}{(1+z)^2},$$

where $g_1 = \dfrac{8s-1}{4(16s-1)}$.

Thus $$(1+z)^2\, 100\text{E} = \lambda_1(1+g_1 z) + 100 - \lambda_1.$$

and $$z^2 + \left(2 - \frac{g_1\lambda_1}{100\text{E}}\right) z + 1 - \frac{1}{\text{E}} = 0. \tag{12}$$

This is a quadratic which determines $z$, and thus the values of $e_1$ and $e$ can at once be obtained. The percentage of the allogenic population which is the offspring of first cousin marriage is then given by $\lambda_1 e_1/\text{E}$. The value of this function has been calculated for frequencies of occurrence of the allogenic constituent at chosen intervals from 25 per cent. of the whole population to one case in a million. The results are given in Table II.

Here, again, we observe that, when the recessive element is a very rare one, the likelihood of its possessors being the offspring of first cousins is much greater than the frequency of first cousin marriages would lead us to expect.

The statistics available for the purpose of comparison with these results are unfortunately extremely meagre, and they cannot be regarded as more than approximately accurate.

Miss E. M. Elderton has kindly placed at my disposal her results as to the percentage frequency of consanguinity among the parents of albinos. Miss Elderton surmises that, when in her statistics it is not distinctly recorded that there was no consanguinity between the parents, it is possible that there may have been some relationship.

Hence she obtains two results: firstly, by including all available figures, and, secondly, by excluding those families in which the presence or absence of consanguinity in the parentage is not distinctly recorded.

In the first case she finds that 14 per cent., and in the second that 21·6, of albinos are the offspring of first cousin and double cousin marriages.

Now, albinism is usually regarded as arising from a recessive Mendelian factor, although strict proof of this is as yet wanting. Assuming that it is such a Mendelian unit character, the above results can be applied to it.

Prof. Pearson estimates that albinism occurs in about 1 individual in 20,000. With this frequency, we see from Table II that if the general rate of first cousin marriage is about 1 or 2 per cent., the percentage of albinos that arises from first cousin marriages, as found from calculation, is of about the same magnitude as that found from direct statistics. If the marriages of first cousins form a greater proportion than 1 or 2 per cent. of all marriages, then the calculated result becomes too great.

It is, however, quite impossible as yet to say definitely whether the percentage of first cousin marriages largely exceeds 2 or not. In any case the approximate character of the other data precludes us from obtaining more than a rough test as to whether the calculated and observed results are of more or less of the same order of magnitude.

The only other numerical test obtainable has been given by the data as to deaf-mutism. In the American Census of 1890 it was found that there were 659 congenitally deaf persons in 10,000,000 inhabitants, or, about 1 person in 15,000. It is probable that most of these were deaf mutes.

Miss Elderton finds that 8 per cent. of deaf mutes are the offspring of first cousin parents. The calculated result for 1 per cent. of cousin marriage lies between 8·8 and 11·9 for $16s = 4$, and between 12·6 and 17·1 for $s = \infty$. Here again the calculated result seems to be somewhat too high. Both in this case, however, and in that of albinism it may very well be that there is a selective infantile death rate, unfavourable to albinos and deaf mutes, and that, consequently, the conditions of albinism and deaf-mutism are really of more frequent occurrence than would appear from a count of the population at any single instant.

If this is so, Table II shows that there will be a great reduction in the calculated number of deaf mute and albinotic persons who are the offspring of a consanguineous parentage.

Another point to be noticed is that Table II has been formed on the assumption that first cousin marriage alone has a greater rate of production of allogenic characters than the non-inbreeding population, whereas some of the excess rate is due to second cousin and other consanguineous marriages. As a matter of fact, if $\lambda_2$ be the second cousin marriage rate, and $g_2$ the proportion of allogenic element produced by it, and if $\lambda_3$, $g_3$, be the same functions for third cousin marriages and so on, then equation (12) becomes

$$z^2+z\left(1-\frac{g_1\lambda_1+g_2\lambda_2+g_3\lambda_3+\dots}{100\mathrm{E}}\right)+1-\frac{1}{\mathrm{E}}=0. \qquad (13)$$

and this equation leads to a larger value of $z$ than (12).

Thus (13) determines a smaller value of $e_1$ than (12). Hence if the second and third cousin rates were taken into account the values given in Table II for $\lambda_1 e_1/\mathrm{E}$ would to some extent be reduced.

The second and third cousin marriage rates being almost wholly unknown, it has not been feasible to allow for them. But part of the discrepancy noted between calculation and observation can be explained in this way.

9. Finally, it is important to tabulate the relative rates of production of allogenic element by first cousin parents and by the non-related parents, when there is a given frequency of occurrence of the allogenic element in the population at large. The percentage of first cousin marriage is again taken to have values from 1 to 5.

The rates of production of the allogenic element by first cousin and non-consanguineous marriages are respectively $(1+g_1z)/(1+z^2)$ and $1/(1+z^2)$, where $z$ is given by equation (12). The relative rate of production is thus simply $1+g_1z$, where $g_1=\dfrac{8s-1}{4(16s-1)}$. This has been calculated for $16s=4$ and $s=\infty$, and the results are given in Table III.

This table shows that while for a pure recessive which occurs in more than 1 per cent. of the general population, a first cousin marriage is at most not more than twice as likely to produce this constituent in its offspring than a non-consanguineous marriage, in the case of a very rare recessive constituent with a frequency, say, of 1 in 20,000, first cousins are from

Table II.—Showing the Percentage of Individuals of Allogenic Constitution who are the Offsprings of First Cousins.

| Proportion of allogenic element in the general population. | | 25 per cent. | 10 per cent. | 5 per cent. | 4 per cent. | 3 per cent. | 2 per cent. | 1 per cent. | 1/1000 | 1/10,000 | 1/20,000 | 1/1,000,000 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 per cent. of cousin marriage............ | $16s = 4$ | 1·08 | 1·18 | 1·29 | 1·33 | 1·39 | 1·50 | 1·74 | 3·53 | 8·8 | 11·92 | 55·8 |
| | $s = \infty$ | 1·12 | 1·27 | 1·43 | 1·49 | 1·59 | 1·75 | 2·12 | 5·02 | 12·6 | 17·1 | 69·6 |
| 2 per cent. of cousin marriage............ | $16s = 4$ | 2·16 | 2·36 | 2·56 | 2·66 | 2·78 | 2·99 | 3·46 | 6·88 | 16·9 | 22·4 | 78·0 |
| | $s = \infty$ | 2·25 | 2·52 | 2·85 | 2·97 | 3·17 | 3·47 | 4·18 | 9·22 | 23·5 | 30·9 | 87·6 |
| 3 per cent. of cousin marriage............ | $16s = 4$ | 3·26 | 3·51 | 3·84 | 3·97 | 4·16 | 4·45 | 5·16 | 10·1 | 24·6 | 31·6 | 88·2 |
| | $s = \infty$ | 3·37 | 3·79 | 4·26 | 4·45 | 4·73 | 5·19 | 6·24 | 13·5 | 33·0 | 42·4 | 93·5 |
| 4 per cent. of cousin marriage............ | $16s = 4$ | 4·32 | 4·69 | 5·10 | 5·27 | 5·52 | 5·92 | 6·94 | 13·3 | 30·9 | 39·6 | 92·6 |
| | $s = \infty$ | 4·48 | 5·03 | 5·66 | 5·92 | 6·28 | 6·88 | 8·24 | 17·6 | 41·3 | 53·9 | 96·3 |
| 5 per cent. of cousin marriage............ | $16s = 4$ | 5·41 | 5·83 | 6·36 | 6·58 | 6·88 | 7·40 | 8·55 | 16·3 | 36·9 | 46·7 | 95·1 |
| | $s = \infty$ | 5·60 | 6·28 | 7·04 | 7·35 | 7·80 | 8·55 | 10·2 | 21·6 | 48·4 | 59·5 | 97·8 |

Table III.—Showing the Relative Rate of Production of Allogenic Constituent by First Cousin and Non-consanguineous Marriage.

| | Frequency in population at large. | 25 per cent. | 10 per cent. | 5 per cent. | 4 per cent. | 3 per cent. | 2 per cent. | 1 per cent. | 1/1000. | 1/10,000. | 1/20,000. | 1/1,000,000. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $16s = 4.$ | 1 per cent. of cousin marriage ... | 1·08 | 1·18 | 1·29 | 1·33 | 1·40 | 1·51 | 1·75 | 3·56 | 9·64 | 13·4 | 125 |
| | 2 ,, ,, ... | 1·08 | 1·18 | 1·29 | 1·33 | 1·40 | 1·51 | 1·76 | 3·60 | 9·97 | 14·2 | 179 |
| | 3 ,, ,, ... | 1·08 | 1·18 | 1·29 | 1·33 | 1·40 | 1·51 | 1·76 | 3·64 | 10·35 | 14·9 | 238 |
| | 4 ,, ,, ... | 1·08 | 1·18 | 1·29 | 1·34 | 1·40 | 1·51 | 1·76 | 3·69 | 10·74 | 15·8 | 302 |
| | 5 ,, ,, ... | 1·08 | 1·18 | 1·29 | 1·34 | 1·40 | 1·51 | 1·76 | 3·72 | 11·15 | 16·6 | 367 |
| $s = \infty.$ | 1 per cent. of cousin marriage... | 1·12 | 1·27 | 1·43 | 1·50 | 1·60 | 1·76 | 2·13 | 4·90 | 14·1 | 20·2 | 226 |
| | 2 ,, ,, ... | 1·13 | 1·27 | 1·44 | 1·50 | 1·60 | 1·77 | 2·14 | 4·98 | 15·0 | 21·9 | 357 |
| | 3 ,, ,, ... | 1·13 | 1·27 | 1·44 | 1·50 | 1·60 | 1·77 | 2·15 | 5·06 | 15·9 | 23·8 | 498 |
| | 4 ,, ,, ... | 1·13 | 1·27 | 1·44 | 1·51 | 1·60 | 1·77 | 2·15 | 5·14 | 16·8 | 24·9 | 649 |
| | 5 ,, ,, ... | 1·13 | 1·27 | 1·44 | 1·51 | 1·61 | 1·78 | 2·16 | 5·22 | 17·8 | 27·0 | 801 |

13 to 28 times more likely to have this pure recessive in their offspring than are non-related couples.

Were Mendelism completely established as a theory of reproduction applicable to man, and were it shown that good qualities were absolute dominants and bad qualities always recessive, the above result might legitimately be used to show that first cousin marriages are undesirable. But this is not so, and, in particular, it seems probable that dominance is not complete, and that there may be some dominant harmful characters and some useful recessive ones.

### *General Conclusions and Limitations.*

10. (i) The not unimportant result has been proved that the more infrequent a pure recessive factor is in any simple stable Mendelian population breeding at random, then this pure recessive is less and less likely to occur in the offspring of any type of marriage, whether consanguineous or not, though at the same time it becomes relatively more and more likely to occur in the offspring of related pairs. In other words, with a less and less frequently occurring pure recessive, the decrease in the appearance of a pure recessive in the offspring of related couples is not so rapid as it is in the case of non-related pairs.

Precisely the same conclusion applies to the production of the pure dominant. Further, it has been shown that the relative frequency of the appearance of the allogenic constituent in the offspring of related pairs diminishes by about one-half for each grade of cousinship. Hence, if anything can be urged against first cousin marriages as productive of patent recessive qualities, the argument applies with less and less force to cousin marriages of higher grades.

(ii) The following general proposition throws a good deal of light on this problem of inbreeding in a simple Mendelian population, namely :—

The offspring of any system of inbreeding, provided there is no selective marriage or death rate or differential fertility, can be expressed in the form

$$p^2(\mathrm{AA})+2pq(\mathrm{A}a)+q^2(aa)+f(p,q)[(\mathrm{AA})-2(\mathrm{A}a)+(aa)].$$

That is to say, the offspring of consanguineous marriage can be expressed as a sample of the general population, together with a part due to the inbreeding factor $(\mathrm{AA}) - 2(\mathrm{A}a) + (aa)$.

To show this, let the offspring of any system of inbreeding consist of a regrouping of the allelomorphs A and $a$, such that the term $\mathrm{L}(\mathrm{AA}) + \mathrm{M}(\mathrm{A}a) + \mathrm{N}(aa)$ is added to the general population. L, M, N, are functions of $p, q$.

Now, in a population stable both as regards type and number, the total quantity of allelomorphs, whether dominant or recessive, must remain unaltered. Hence we have at once,

$$2\mathrm{L}+\mathrm{M} = 0 \quad \text{and} \quad 2\mathrm{N}+\mathrm{M} = 0.$$

Putting $\mathrm{L} = f(p, q)$, the result above is obtained at once.

In the case of the children of brother-sister marriages $f(p, q) = pq/4$; for the children of first cousins, for $s = \infty$, $f(p, q) = pq/8$, and so on.

Inbreeding thus accentuates both the pure dominant strain and the pure recessive in any stock to the same extent, and at the expense of the hybrid element.

The already noted agreement between the actually observed somatic correlations and the theoretical Mendelian gametic correlations appears to negative the existence of absolute dominance. Suppose, then, we are dealing with a harmful recessive characteristic, the result of inbreeding is that for every individual with patent evil added to the community, a useful individual of pure dominant type is also added, and two individuals of neutral type are got rid of. It is quite conceivable that this process might be a gain to the community, owing to a selective action on the patent evil.

It would, of course, in the present state of our knowledge, be impossible to insist on this view: but, at the same time, it must be pointed out that much of the argument against inbreeding fails if the phenomenon of dominance be not complete. In the particular case in which the heterozygote has a mean "utility" between the utilities of the two homozygotes inbreeding is neither advantageous nor the reverse.

(iii) Further, as Bateson* has pointed out, it may be that some valuable qualities are recessive in character.

In that case, and especially for a very rare recessive, as the preceding analysis shows, inbreeding will serve a useful purpose in bringing the recessive quality to light.

(iv) The analysis has dealt solely with matings between collaterals of the same rank. It may, however, be pointed out that where the marriages of first cousins once removed are considered, these should probably be regarded in the same light as second cousin marriages, so far as resemblance can be used as a test. For, just as it has been found that first cousins resemble each other to about the same extent as uncle and nephew or aunt and nieces, so it appears likely that the correlation between first cousins once removed will be equal to the correlation between second cousins. But whether this equality of correlation exists, and whether the somatic resemblance can be used as a test for predicting the consequences of inbreeding, are points which must be left for future elucidation.

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* 'Mendel's Principles of Heredity,' 1909.

(v) Some of the limitations of the method of this paper have been pointed out, and others are obvious. The extension to the case where more than one pair of allelomorphs is considered might conceivably be valuable when more data have been collected, and should not prove difficult.

In particular, a method which assumes the absence of selective mating and ignores the existence of differential fertility can claim no finality. It is hoped, however, that some of the conclusions are fairly exact deductions from the simple Mendelian theory as it stands at the present time.

In conclusion, I wish to thank Prof. Pearson for the help noted in the paper, and for much stimulating criticism.

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# *Cancerous Ancestry and the Incidence of Cancer in Mice.*

By J. A. Murray, M.D., B.Sc., Imperial Cancer Research Fund.

(Communicated by Prof. J. Rose Bradford, Sec. R.S. Received March 22,—Read May 4, 1911.)

The purpose of these experiments has been the collection of data sufficiently abundant and accurate to determine whether an enhanced liability to cancer is transmitted in the case of mice from parents to offspring. In a preliminary note in 1909* a short account was given of the manner in which these experiments have been conducted.

The animals have all been housed and fed in a uniform manner in one room. They have been kept in large cages, which have been cleaned regularly, and the environment has been as uniform as it has been possible to make it. During the past five years nearly 1600 animals have been bred, the two sexes contributing approximately equal numbers. Of them, 562 females which have lived for six months or more form the materials of the present paper. The incidence of the disease is so dependent on the age and sex of the animals that, in order to get comparable groups, only mice of the same sex and of approximately the same age may be reckoned together.

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* E. F. Bashford and J. A. Murray, "The Incidence of Cancer of the Mamma in Female Mice of Known Age," 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 310.

They have been arranged in age-periods of three months' duration, this being the shortest interval which gives reasonably large figures in each group.

From the pathological standpoint the data are practically perfect. All the animals which did not present a tumour during life were carefully examined for tumours after death, and it is scarcely possible that any growth of considerable size has escaped being examined microscopically and recorded. The mice in which tumours were discovered during life have been kept under observation till death. The tumours have been examined microscopically in every case, and only those which were undoubtedly malignant are reckoned in the tables and ancestries.

The figures refer to females only. The number of cases of malignant new growths in males bred in the laboratory is so small that a statistical study of their frequency could not give useful results, nor do the males which developed new growths appear in the ancestry of the females at present under consideration.

The tables show the ratio which deaths from cancer, and more especially cancer of the mamma, bear to deaths from all causes at each of seven three-monthly age-periods for female mice over a number of years. The age-period in which mice dying of diseases other than cancer are entered is given by the age at death. The mice which have died of cancer are entered in the age-period embracing their ages at the time the existence of a tumour was discovered, and not in that embracing their age at death.* This basis for the age groups has been chosen instead of the actual age at death, because the latter varies with the exigencies of other experiments having no direct connection with the statistical studies. In order to include cancerous mice still living and so increase the volume of the data it was necessary to increase the non-cancerous totals also, by including living non-cancerous mice. These have been included in the age-periods embracing their ages on a selected day (in the case of the present tables, October 24, 1910).

Table I, giving this distribution, and discriminating between cancer of the mamma and cancer of other organs, shows a rapidly increasing proportion of deaths from cancer commencing after six months had passed, attaining a maximum in the three-monthly period ending at 18 months, and then diminishing till, in mice over 24 months old, the frequency is

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* In the earlier paper, one mouse was recorded as exactly six months old when the tumour developed. Actually, the tumour was discovered at the age of six months and seven days, and the mouse is therefore entered in the six to nine months age-period in the present tables.

TABLE I. (October 24, 1910.) Female Mice.

| Age (months) ... | 0–3 | –6 | –9 | –12 | –15 | –18 | –21 | –24 | Over 24 | Total. |
|---|---|---|---|---|---|---|---|---|---|---|
| No tumour— | | | | | | | | | | |
| Living | — | — | 16 | 7 | 7 | 9 | 16 | 9 | 21 | — |
| Dead | — | — | 79 | 85 | 63 | 56 | 41 | 37 | 24 | — |
| Tumour mice— | | | | | | | | | | |
| Organs other than mamma | — | — | — | 1 | 2 | 2 | 2 | 1 | 3 | — |
| Mamma | — | — | 5 | 11 | 16 | 26 | 10 | 8 | 5 | — |
| Total | — | — | 100 | 104 | 88 | 93 | 69 | 55 | 53 | 562 |
| Per cent. of mammary cancer | — | — | 5·0 | 10·6 | 18·2 | 28·0 | 14·5 | 14·0 | 9·4 | 14·4 |

barely twice that found in mice under nine months old. Similar figures for the human female give a corresponding curve.

Tables II and III were made after a preliminary distribution of the mice in groups, according as the first cancerous ancestor occurred in the 1st, 2nd, 3rd,... ascending generation, had shown that the majority of the cases of cancer occurred in mice in which either the mother, or a grandmother, or all three, developed cancer.

TABLE II. (24th October, 1910.) Female Mice of Recent Cancerous Ancestry. (Mother, one or both grandmothers, or all three cancerous.)

| Age (months) ... | 0–3 | –6 | –9 | –12 | –15 | –18 | –21 | –24 | Over 24 | Total. |
|---|---|---|---|---|---|---|---|---|---|---|
| No tumour— | | | | | | | | | | |
| Living | — | — | 9 | 7 | 6 | 8 | 7 | 4 | 6 | — |
| Dead | — | — | 49 | 48 | 39 | 28 | 22 | 20 | 18 | — |
| Tumour mice— | | | | | | | | | | |
| Organs other than mamma | — | — | — | 1 | 2 | 2 | 1 | — | 1 | — |
| Mamma | — | — | 4 | 7 | 15 | 18 | 10 | 5 | 3 | — |
| Total | — | — | 62 | 63 | 62 | 56 | 40 | 29 | 28 | 340 |
| Per cent. of mammary cancer | — | — | 6·5 | 11·1 | 24·2 | 32·1 | 25·0 | 17·2 | 10·7 | 18·2 |

Table II shows the proportions in which mice of recent cancerous ancestry, in this limited sense, died of cancer at the different age-periods. Table III is to be compared with Table II, and gives the same distribution for the remaining mice in which the cancerous ancestors are more remote. The curves in fig. 1 show the differences between the percentage of deaths from cancer in the two groups at successive age-periods. The two percentage curves differ very little at the early and at the final periods, but diverge in the middle.

TABLE III. (24th October, 1910.) Female Mice of Remote Cancerous Ancestry. (No cancer in mother or grandmothers.)

| Age (months) ... | 0-3 | -6 | -9 | -12 | -15 | -18 | -21 | -24 | Over 24 | Total. |
|---|---|---|---|---|---|---|---|---|---|---|
| No tumour— | | | | | | | | | | |
| Living ............... | — | — | 7 | — | 1 | 2 | 9 | 5 | 15 | — |
| Dead .................. | — | — | 30 | 37 | 24 | 28 | 19 | 17 | 6 | — |
| Tumour mice— | | | | | | | | | | |
| Organs other than mamma ......... | — | — | — | — | — | — | 1 | 1 | 2 | — |
| Mamma ............ | — | — | 1 | 4 | 1 | 8 | — | 3 | 2 | — |
| Total............ | — | — | 38 | 41 | 26 | 37 | 29 | 26 | 25 | 222 |
| Per cent. of mammary cancer ...... | — | — | 2·6 | 9·8 | 3·8 | 21·6 | 0·0 | 11·5 | 8·0 | 8·6 |

Two other curves (fig. 2) constructed in the same way with slightly different limits to the age-period show the same features, except that in the highest age-group (over 25 months in this case) the percentage of deaths from cancer, in the mice of cancerous ancestry, falls just below that in the non-cancerous group (1 in 15 as compared with 1 in 14).

The results of the two distributions agree very closely, and strongly suggest a real difference inherent in the data. Consideration of the factors relegating an animal to one or the other of these two groups (ancestry cancerous, or ancestry non-cancerous) enhances the importance of the difference between them. On the one hand, the cancerous group (Table II) includes many mice with only slight hereditary taint. On the other hand, the mice with non-cancerous ancestry (Table III) are a mixed group; they comprise a certain number which, but for the accident of the early death of parent and grandparents, would have to be added to the group with cancerous ancestry. When a comparison is made between the ancestors of the tumour mice of the non-cancerous group and the ancestors of those which died free from cancer, the ancestors of the tumour mice died in greater proportion in the early age-periods. Hence, if there could be eliminated from the non-cancerous group those mice which are included in it because of the early death of their female parents and grandparents, tumour mice would be transferred from the non-cancerous group to that with

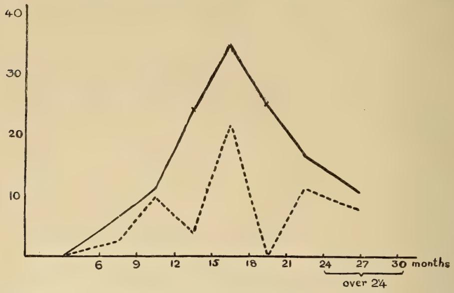

FIG. 1.—Percentage of deaths from mammary carcinoma to deaths from all causes at successive three-monthly age-periods in female mice of recently cancerous ancestry (mother, grandmothers) ——, compared with the same ratio in female mice having more remote cancerous ancestry (mother and grandmothers non-cancerous) - - - - -.

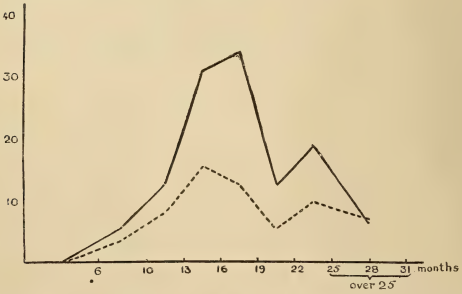

FIG. 2.—The same comparison as in fig. 1, with different limits to the three-monthly age-periods (second period, four months). As in fig. 1, the frequency of the deaths from cancer in the cancerous group exceeds that of the non-cancerous group at all periods except the last.

recent cancerous heredity, in greater proportion than mice which did not develop cancer. In fact, the difference between the two groups is a minimum difference, and it should be possible, by continued selective mating, to breed two strains of mice with a still greater difference in their liability to cancer.

In conclusion, it is well to consider the importance of these results in the light of the comparative pathology of cancer. Investigations of the most diverse kind on man and animals show that the actual initiation of cancer is, in many forms of the disease, a terminal phase of a long-continued process of localised chronic irritation. Even in mice in whose ancestry cancer is absent, cancer may arise in consequence of such irritation, particularly when they attain extreme old age, in large numbers, and a diminished predisposition to the disease would merely effect a diminution in the number of individuals attacked as compared with a corresponding number of individuals with inherited liability similarly irritated. The phenomena of the experimental production of sarcoma by transformation of the stroma of transplanted tumours of a particular strain in nearly all animals, however, indicate that the diminished liability is never likely to become absolute in practice. Conversely, it should be possible by shielding individuals, even of highly susceptible stock, from chronic irritation of specific tissues to diminish considerably the incidence of cancer of these tissues amongst them.

Other investigations have shown that a constitutional condition favouring the *growth* of cancer and accounting for its incidence is not present in mice which suffer spontaneously from the disease. The determining factors are those which *initiate* cancerous proliferation, and it is highly probable that the predisposing condition which is transmitted is some peculiarity of the cells of the tissues in which cancer develops, of such a kind that, under the wear and tear of life, the regenerative and proliferative changes which accompany the inception of the disease are more prone to occur, or take place with greater intensity. The present observations harmonise with the conclusion drawn from other lines of work, that cancer always arises *de novo* in the organism attacked by a transformation of the ordinary tissue elements, and lend no support to the view that groups of cells outside the anatomical and physiological nexus of the organism from an early period in the ontogeny form the physical basis of the development of malignant new growths.

The figures have also been submitted to mathematical analysis, involving determination of the standard errors of the differences between the cancerous and non-cancerous groups. Taking the crude data, the actual percentages amongst all the offspring are:—

| | |
|---|---|
| Ancestry cancerous | 18·2 per cent. |
| Ancestry non-cancerous | 8·6 „ |
| Difference | 9·6 „ |

these percentages being based on 340 and 222 cases respectively. When a correction is made for the varying age-distributions of the two groups by calculating corrected percentages based on the age-distribution of all mice and reducing the numbers to the corresponding proportions per thousand, the difference is merely slightly increased from 9·6 to 9·8 per cent. The standard error of this difference is 2·96. The difference is 3·3 times the standard error, and the chance of its occurring as a mere fluctuation of random sampling only about 1 in 1000.

The following are the differences for the separate age-classes, with their standard and probable errors:—

| Age. | Difference. | Standard error. | Probable error* = 0·6745 standard error. |
|---|---|---|---|
| – 9 | 3·9 | 4·49 | 3·03 |
| –12 | 1·3 | 6·18 | 4·17 |
| –15 | 20·4 | 9·02 | 6·08 |
| –18 | 10·5 | 7·98 | 5·38 |
| –21 | 25·0 | 8·59 | 5·79 |
| –24 | 5·7 | 9·51 | 6·41 |
| 24– | 2·7 | 8·03 | 5·42 |

* The probable error is the fluctuation of sampling that will be as often exceeded as not.

It will be seen that four of the seven differences exceed their probable errors, but the only differences that do so at all considerably are those for the three central age-groups. The difference between the two groups is almost certainly significant, *i.e.* not due to mere fluctuations of sampling.

## *Immunisation by Means of Bacterial Endotoxins.*

By R. Tanner Hewlett, M.D.

(Communicated by Prof. W. D. Halliburton, F.R.S. Received March 28,—
Read May 4, 1911.)

In a former paper* it was shown that the intra-cellular constituents of bacterial cells, bacterial endotoxins, possess a considerable capacity for increasing the opsonising action of the serum of healthy rabbits. In the present paper, the action of bacterial endotoxins in immunising against injections of the corresponding living organisms has been investigated. Smallman, in an investigation upon the active immunisation of experimental animals with typhoid cell juices,† found that the fresh fluid cell juice and also the dried juice immunised guinea-pigs against injections of living typhoid culture. The endotoxins were prepared in the manner described in the previous paper. Guinea-pigs were the experimental animals employed throughout.

The results obtained are summarised in the following sections:—

### *Immunisation against the* Bacillus Typhosus.

*Series I.*—Fifteen guinea-pigs were inoculated on May 14, 1909, each with 1 mgrm. of typhoid endotoxin prepared on May 12, 1909.

*Series II.*—Fifteen guinea-pigs were inoculated on the same date (May 14) each with 0·1 mgrm. of typhoid endotoxin (same preparation).

*Series III.*—Fifteen guinea-pigs were inoculated on May 26, 1909, each with 0·01 mgrm. of typhoid endotoxin (same preparation).

*Series IV.*—Sixteen guinea-pigs were inoculated on May 21, 1909, each with 1 c.c. of Messrs. Burroughs and Wellcome's anti-typhoid vaccine (equivalent to 1,000,000,000 bacilli).

All the guinea-pigs, at the time of inoculation, weighed 250 to 300 grm.

Of *Series I*, one died on May 21, two on May 22, and one on May 28, leaving 11 animals for experiment.

Of *Series II*, one died on May 22, and another on June 1, leaving 13 animals for experiment.

Of *Series III*, one died on June 2, leaving 14 animals for experiment.

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 325. See also 'Roy. Soc. of Med. Proc.,' April, 1910 (Pathological Section), p. 165, in which the effect of injections of the various tuberculins and of tubercle endotoxin on the opsonising action of the serum of healthy rabbits is considered.

† 'R.A.M.C. Journ.,' April, 1905, vol. 4, No. 4, p. 424.

Of *Series IV*, none died, leaving 16 animals for experiment.

All the surviving animals were subsequently inoculated with living typhoid culture intra-peritoneally at varying dates, control animals being similarly inoculated at the same time to test the virulence of the culture. The controls weighed 300 to 360 grm. The results obtained are given in the following tables :—

*Series I*.—Inoculated with 1·0 mgrm. of typhoid endotoxin on May 14, 1909.

Table I.—Inoculated with Living Culture on June 18, July 17, and July 21, 1209.

| Reference No. of guinea-pig. | Date of inoculation. | Amount of culture. | Result. |
|---|---|---|---|
| 21 | June 18 | 5 M.L.D.'s | *Lived.* |
| 2 | ,, | 2 ,, | ,, |
| 23 | ,, | 2 ,, | ,, |
| Control | ,, | 5 ,, | + Dead, June 19. |
| ,, | ,, | 2 ,, | *Lived.* |
| ,, | ,, | 1 M.L.D. | + Dead, June 19. |
| 36 | July 7 | 5 M.L.D.'s | *Lived.* |
| 25 | ,, | 5 ,, | ,, |
| 5 | ,, | 5 ,, | ,, |
| 16 | ,, | 5 ,, | + Dead, July 8. |
| Control | ,, | 5 ,, | + ,, ,, |
| ,, | ,, | 5 ,, | + ,, ,, |
| ,, | ,, | 2½ ,, | *Lived.* |
| ,, | ,, | 1·2 ,, | + Dead, July 8. |
| 33 | July 21 | 8 ,, | *Lived.* |
| 24 | ,, | 8 ,, | ,, |
| 19 | ,, | 8 ,, | ,, |
| 28 | ,, | 8 ,, | ,, |
| Control | ,, | 8 ,, | + Dead, July 22. |
| ,, | ,, | 4 ,, | + ,, ,, |
| ,, | ,, | 2 ,, | + ,, ,, |

M.L.D. = minimum lethal dose.

*Series II.*—Inoculated with 0·1 mgrm. of Typhoid Endotoxin on May 14, 1909.

Table II.—Inoculated with Living Culture on June 18, July 7, July 21, and July 28, 1909.

| Reference No. of guinea-pig. | Date of inoculation. | Amount of culture. | Result. |
|---|---|---|---|
| 27 | June 18 | 5 M.L.D.'s | *Lived.* |
| 10 | ,, | 2 ,, | ,, |
| 12 | ,, | 2 ,, | ,, |
| 17 | July 7 | 5 ,, | ,, |
| 22 | ,, | 5 ,, | ,, |
| 30 | ,, | 5 ,, | ,, |
| 32 | ,, | 5 ,, | ,, |
| 34 | July 21 | 8 ,, | ,, |
| 20 | ,, | 8 ,, | ,, |
| 1 | ,, | 8 ,, | + Dead, July 22. |
| 8 | ,, | 8 ,, | *Lived.* |
| 14 | July 28 | 8 ,, | ,, |
| 31 | ,, | 8 ,, | ,, |
| Control | ,, | 8 ,, | + Dead, July 29. |
| ,, | ,, | 4 ,, | + ,, ,, |

The controls to the animals inoculated on June 18, July 7, and July 21 were the same as those in Table I.

*Series III.*—Inoculated with 0·01 mgrm. of Typhoid Endotoxin on May 26, 1909.

Table III.—Inoculated with Living Culture on June 18, July 7, July 21, and July 28, 1909.

| Reference No. of guinea-pig. | Date of inoculation. | Amount of culture. | Result. |
|---|---|---|---|
| 61 | June 18 | 5 M.L.D.'s | *Lived.* |
| 67 | ,, | 2 ,, | ,, |
| 53 | ,, | 2 ,, | ,, |
| 46 | July 7 | 5 ,, | ,, |
| 58 | ,, | 5 ,, | ,, |
| 62 | ,, | 5 ,, | ,, |
| 68 | ,, | 5 ,, | ,, |
| 44 | July 21 | 8 ,, | + Dead, July 22. |
| 50 | ,, | 8 ,, | + ,, ,, |
| 55 | ,, | 8 ,, | *Lived.* |
| 63 | ,, | 8 ,, | + Dead, July 22. |
| 64 | ,, | 8 ,, | + ,, ,, |
| 40 | July 28 | 8 ,, | + ,, July 29. |
| 52 | ,, | 8 ,, | *Lived.* |

The controls to the animals inoculated on June 18, July 7, and July 21 were the same as those in Table I, and on July 28 as in Table II.

*Series IV.*—Inoculated with 1 c.c. of typhoid vaccine (1,000,000,000 bacilli) on May 21, 1909.

Table IV.—Inoculated with Living Culture on June 18, July 7, July 21, and July 28, 1909.

| Reference letter of guinea-pig. | Date of inoculation. | Amount of culture. | Result. |
|---|---|---|---|
| C | June 18 | 5 M.L.D.'s | *Lived.* |
| CC | " | 2 " | + Dead, June 19. |
| FF | " | 2 " | *Lived.* |
| A | July 7 | 5 " | " |
| K | " | 5 " | " |
| V | " | 5 " | " |
| Z | " | 5 " | " |
| BB | July 21 | 8 " | + Dead, July 22. |
| DD | " | 8 " | + " " |
| KK | " | 8 " | + " " |
| H | " | 8 " | *Lived.* |
| N | " | 8 " | + Dead, July 22. |
| B | July 28 | 8 " | + Dead, July 29. |
| HH | " | 8 " | + " " |
| O | " | 8 " | + " " |
| P | " | 8 " | Ill, but recovered. |

The controls to the animals inoculated on June 18, July 7, and July 21 were the same as those in Table I, and on July 28 as in Table II.

*Summary of Results obtained with Typhoid Endotoxin.*—The experiments detailed in Tables I, II, and III indicate that injections of 1, 0·1, and 0·01 mgrm. of typhoid endotoxin confer considerable protection against subsequent injections of living typhoid bacilli. With amounts of 1 and 0·1 mgrm. of endotoxin the protective power was maintained for a period of nearly 11 weeks. With 0·01 mgrm. of endotoxin the protective power appeared to be maintained for six weeks, but did not seem to be manifest after eight and nine weeks; but it is to be noted that in the two last instances the test dose of typhoid bacilli was larger than that employed previously. As regards the typhoid vaccine the results obtained with it correspond with those obtained with the lowest dose of endotoxin, viz., 0·01 mgrm.

### *Immunisation against the* Bacillus diphtheriæ.

The diphtheria endotoxin was prepared by growing a virulent diphtheria bacillus on the surface of blood-agar for 48 hours, collecting the growth, well washing twice with physiological salt solution by centrifuging in order to remove adherent toxin, and grinding, etc., in the usual way. The endotoxin solution was prepared on June 17, 1909.

*Series I.*—Six guinea-pigs, each weighing 230 to 240 grm. were inoculated with the diphtheria endotoxin on July 7, 1909, three receiving 1 mgrm. each, and three 0·5 mgrm. each of the endotoxin. One of the guinea-pigs which received 1 mgrm. died on July 12. The five survivors, together with two controls, were each inoculated subcutaneously with 1/10 of a loop of a 24-hour blood-agar culture of virulent diphtheria. The virulence of this strain of diphtheria bacillus had been previously tested on June 25 with the following results (Table V):—

Table V.—Virulence of Diphtheria Culture.

| Reference letter of guinea-pig. | Amount of culture. | Result. |
|---|---|---|
| R | 2 loops | + Dead, June 27 (two days). |
| B | 1 loop | + „ „ 28 (three days). |
| Z | 0·5 loop | + „ „ 28 „ |
| A | 0·1 loop | + „ „ 28 „ |
| P | 0·01 loop | Induration and paresis, July 2. |

One-tenth of a loop therefore contained at least three or four minimal lethal doses.

The result of the inoculation of the animals treated with the diphtheria endotoxin, together with two controls, is given in the following table (Table VI):—

Table VI.—Inoculation of Vaccinated Animals with Culture ($\frac{1}{10}$ loop), July 14, 1909.

| Reference letter of guinea-pig. | Amount of endotoxin. | Result. | |
|---|---|---|---|
| | mgrm. | | |
| O | 1·0 | *Lived.* | The four survivors had ulcers at the seat of inoculation of the culture on July 26. The ulcers were tending to heal and the animals lived on. |
| R | 1·0 | + Dead, July 21 | |
| P | 0·5 | *Lived.* | |
| L | 0·5 | „ | |
| S | 0·5 | „ | |
| SS | Control | + Dead, July 17 | |
| T | „ | + „ „ | |

The results indicate that the diphtheria endotoxin confers considerable protection against the injections of living diphtheria bacilli.

### *Immunisation against the* Vibrio choleræ.

The endotoxin solution was prepared on June 29, 1909. On August 11, 1909, six guinea-pigs (300 to 400 grm. weight) were each inoculated subcutaneously with 0·25 mgrm. On September, 15, 1909, these guinea-pigs were each inoculated intra-peritoneally with one loop of cholera culture, equivalent to about two minimum lethal doses. Three control animals were inoculated at the same time with the culture, two receiving one loop each, the third 0·5 loop.

All the six vaccinated animals survived, but of the controls the two receiving 1 loop of culture were dead on September 16, and the one receiving 0·5 loop was very ill but recovered.

The cholera endotoxin, therefore, protected the animals against living cholera culture.

### *Immunisation against the* Bacillus pestis.

The plague endotoxin was prepared in the usual manner, and was used fresh (24 hours old). Subsequently the animals were inoculated with virulent plague culture. The results are shown in the following tables (Tables VII and VIII):—

*Series I.*—The guinea-pigs were injected subcutaneously with 1 mgrm. of plague endotoxin on August 11, 1909, and, together with controls, were inoculated intra-peritoneally with plague culture on September 15.

Table VII.

| Reference No. of guinea-pig. | Amount of endotoxin. | Amount of culture. | Result. |
|---|---|---|---|
| | mgrm. | | |
| 1 | 1·0 | $\frac{1}{40}$ loop | *Lived.* |
| 2 | 1·0 | ,, | ,, |
| 3 | 1·0 | ,, | ,, |
| 4 | 1·0 | ,, | ,, |
| 5 | 1·0 | ,, | ,, |
| 6 | 1·0 | ,, | ,, |
| Control | Control | ,, | + Dead, Sept. 19. |
| ,, | ,, | ,, | *Lived.* |

The animals which survived were alive and well on October 2, 1909, *i.e.* 17 days after inoculation with the plague culture.

*Series II.*—The plague endotoxin was that prepared on August 10, 1909. The guinea-pigs were injected subcutaneously with plague endotoxin on November 18, and, together with the controls, were inoculated intra-peritoneally with virulent plague culture on December 9, *i.e.* three weeks later.

Table VIII.

| Reference No. of guinea-pig. | Amount of endotoxin. | Amount of culture. | Result. |
|---|---|---|---|
| | mgrm. | | |
| 10 | 2·0 | $\frac{1}{30}$ loop | *Lived.* |
| 12 | 2·0 | ,, | ,, |
| 7 | 1·0 | ,, | ,, |
| 8 | 1·0 | ,, | ,, |
| 9 | 1·0 | ,, | ,, |
| 13 | 0·5 | ,, | ,, |
| 14 | 0·5 | ,, | ,, |
| 15 | 0·5 | ,, | ,, |
| 3 | Control | ,, | + Dead, Dec. 13. |
| 4 | ,, | ,, | + ,, ,, |
| 5 | ,, | ,, | *Lived.* |

The animals which survived were alive December 31, 1909.

On the whole, it would appear that the plague endotoxin does confer considerable protection against living plague bacilli.

*Simultaneous Inoculation with Two and Three Different Endotoxins.*

A few inoculations were simultaneously done with two and three different endotoxins.

The endotoxins were injected subcutaneously on August 11, and the cultures were inoculated intra-peritoneally on September 15, *i.e.* five weeks later. The typhoid endotoxin was prepared on June 29, the cholera endotoxin on June 29, and the diphtheria endotoxin on June 17.

The results are given in the following tables (Tables IX and X).

*Series I.*—Two endotoxins.

Table IX.

| Reference No. of guinea-pig. | Endotoxin, August 11. | Culture, September 15. | Result. |
|---|---|---|---|
| 1 | 0·25 mgrm. typhoid<br>+0·25 ,, cholera | 1 loop cholera | + Dead, September 16. |
| 2 | ,, ,, | 1 ,, ,, | *Lived.* |
| 3 | ,, ,, | 1 ,, ,, | ,, |
| 4 | ,, ,, | 0·5 ,, typhoid | ,, |
| 5 | ,, ,, | 0·5 ,, ,, | ,, |
| 6 | ,, ,, | 0·5 ,, ,, | ,, |
| A | Control | 1 ,, cholera | + Dead, September 16. |
| B | ,, | 1 ,, ,, | + ,, ,, |
| C | ,, | 0·5 ,, ,, | Ill, but recovered. |
| D | ,, | 0·5 ,, typhoid | + Dead, September 16. |
| E | ,, | 0·5 ,, ,, | + ,, ,, |
| F | ,, | 0·25 ,, ,, | + ,, ,, |

*Series II.*—Three endotoxins.

Table X.

| Reference No. of guinea-pig. | Endotoxin, August 11. | Culture, September 15. | Result. |
|---|---|---|---|
| 7 | 0·25 mgrm. typhoid<br>+0·25 „ cholera<br>+0·5 „ diphtheria | 1 loop cholera | + Dead, Sept. 16. |
| 8 | „ „ | 1 „ „ | *Lived.* |
| 9 | „ „ | 0·5 „ typhoid | „ |
| 10 | „ „ | 0·5 „ „ | „ |
| 11 | „ „ | 0·1 „ diphtheria | + Dead, Sept. 17. |
| 12 | „ „ | 0·1 „ „ | + Dead, Sept. 18. |
| 13 | „ „ | 1 „ cholera<br>0·5 „ typhoid | *Lived.* |

The controls for the typhoid and the cholera were the same as in Series I, Table IX. Controls for the diphtheria were as follows: G and H, 0·1 loop diphtheria, were dead on September 17; K, 0·05 loop diphtheria, was dead on September 19.

The results of these few experiments suggest that the simultaneous injection of two different endotoxins does not interfere with the protection conferred by either.

### Trypanosoma brucei.

A few experiments were performed in order to ascertain whether the method employed in the foregoing work is applicable to immunisation against the *Trypanosoma brucei.*

Rats were inoculated, and, when the trypanosome was abundant in the blood, they were killed and the blood was collected. The blood, together with the spleen in *some* instances, was ground in the usual way, and the fluid obtained was injected into healthy rats, which subsequently were inoculated with living *Trypanosoma brucei.* One, or in some cases two and three, injections of the ground material were given, but in no case was any protection obtained.

Other experiments were also performed, using the blood and triturated spleen, which had been subjected to the action of a freezing mixture of ether and solid carbonic acid, without grinding. It was found that this freezing ruptures, and destroys the vitality of, the trypanosomes. Negative results as regards protection were also obtained by this method.

### *General Summary.*

The results obtained in this investigation indicate that typhoid, cholera, diphtheria, and plague endotoxins confer considerable protection against subsequent inoculation with the corresponding living organisms, a protection which, with appropriate doses of the endotoxin, is exerted for at least 11 weeks after the injection of the endotoxin. This result suggests that endotoxins may be of considerable value as protective or prophylactive vaccines. The endotoxin solutions maintain their activity for at least six weeks.

A few inoculations of typhoid and diphtheria endotoxins have been performed in the human subject. The inoculations cause some local reaction at the site of inoculation, consisting of redness, soreness and stiffness of the part, but little general reaction is induced, nor are any ill effects apparent.

I am indebted to Mr. Henry Wellcome for the facilities he has kindly afforded me for carrying out this work at the Wellcome Physiological Laboratories, and my best thanks are due to Mr. E. Thompson for his invaluable assistance in the preparation of the endotoxins.

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# *On a Method of Disintegrating Bacterial and other Organic Cells.*

By J. E. Barnard and R. T. Hewlett.

(Communicated by Prof. W. D. Halliburton, F.R.S. Received March 28,—Read May 4, 1911.)

[Plate 1.]

The methods hitherto available for accomplishing the disintegration of bacteria may be summarised briefly as follows :—The earliest experiment is that of Buchner* for obtaining the intracellular juices of yeast. This method was adopted by Macfadyen, Harris-Morris, and Rowland in their investigations on "Expressed Yeast Cell Plasma," communicated to the Royal Society, June 19, 1900, but they subsequently introduced some modifications and improvements in the method.

Their improved process was to place the yeast cells in mass in a mechanical contrivance, together with a proportion of added silver sand, and to violently agitate the containing vessel. The rapidly succeeding impacts of the yeast cell with the sand particle ruptured the cell wall and caused the contents of the cell to be expelled.

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* 'Berichte d. Deutsch. Chem. Ges.,' 1897, p. 117, and succeeding papers, 1897–1900.

The objection to this method is that a great rise of temperature rapidly takes place unless very efficient means are adopted for cooling; in fact, the whole mass may quickly reach boiling point unless this is efficiently performed. The cooling method they employed was to surround the containing vessel with brine at a temperature of about −5° C., which sufficed to keep the yeast mass at a temperature of about 15° C. Macfadyen and Rowland later discarded this method, and adopted one in which micro-organisms were disintegrated at the temperature of liquid air, and described their method in a paper on "The Intracellular Constituents of the Typhoid Bacillus."* In this apparatus the organisms in mass are placed in a cylindrical metal vessel, which is itself immersed in a vessel of liquid air. The inner container has a conical-shaped bottom, and in this another cone fits which is caused to rotate and is also free to move vertically. On placing the mass of bacterial or other cells in the container, the cells are rendered extremely brittle by the low temperature of the surrounding liquid air. The cone is caused to rotate inside the container and at the same time to move up and down, engaging at each rise and fall a number of the cells between the bottom of the containing pot and itself. The result is that each time a proportion of them is fractured. The sequence of operations is continued until the micro-organisms are found on microscopical examination to be disintegrated. On the completion of the process the temperature of the mass is allowed to rise, salt solution is added if necessary, and the suspension is then centrifugalised so that the cell bodies and any metallic contamination are removed. The amount that may be dealt with by this method is not large, varying usually from 0·5 to 1 grm., and the time required for the complete disintegration of such a quantity varies from one and a half to two hours.

Subsequent experiments have shown that while it is necessary to maintain the material at a low temperature to ensure the brittleness of the cell and to prevent chemical change during the process, such a low temperature as that of liquid air is not essential.

It appears to the writers that the conditions to be fulfilled in designing an efficient machine for the disintegration of micro-organisms are as follows:—

1. The grinding should be effected in a manner which is, as far as possible, frictionless, so that the risk of rise of temperature and consequent chemical change is avoided so far as possible, even apart from any extraneous cooling arrangement.

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* 'Centralblatt für Bakteriologie,' 1903, No. 8.

2. Approximately, every micro-organism or cell should, sooner or later, be brought with certainty under the influence of the grinding action, so that the number of whole cells remaining is a minimum.

3. The containing vessel in which the grinding action takes place must be so effectually enclosed that during the process of disintegration no cells have any opportunity of escaping. This applies particularly to pathogenic organisms.

4. The appliance must be such that an efficient cooling arrangement may be adopted, and, if necessary, a temperature of 15° to 20° C. below freezing maintained at the actual point at which the grinding action takes place.

5. The action presumably requiring to go on in metallic containing vessels, it should be provided that the actual mechanical disintegration of metal between the grinding surfaces should be as little as possible.

These conditions are in the main complied with in the apparatus to be described (figs. 1 and 2).

The containing vessel consists of a phosphor-bronze body A, in which a number—usually five—of hardened steel balls, B, are placed. The shape of the containing vessel is such that when these balls are at its periphery they accurately fit the inner side of the vessel. The diameter of the vessel may conveniently be slightly less than the sum of the diameters of three balls. The balls are evenly distributed round the pot by means of a cage C, and, during the time they are running, this cage ensures that they are equidistant and do not collide one with another. At the centre of the metal pot is a steel cone D, which is of such a size that it keeps the balls in their proper position, in close contact with the periphery of the containing vessel. The vessel is closed by a screw cap E, through which the steel cone passes, and in which it is free to rotate. Over the whole of this a metal cylinder F is placed, and is screwed down, completely sealing the upper opening in the metal pot. In the top of this metal cylinder a steel bearing G is placed, which has freedom of movement in a vertical direction, but is kept down on the top of the steel cone by the action of a spring. It therefore follows that when this metal cylinder is screwed down, the steel cone is pressed down on to the balls, and the balls are in their turn forced out to the periphery of the metal pot. The whole appliance is mounted on a cone H, which is the upper end of a shaft passing through the base plate; on the lower end of the shaft is a grooved wheel K, by which the apparatus may be rotated.

The grinding action is intended to take place between the steel balls contained in the metal pot and the interior surface of the pot, but it is evident that, so long as the whole appliance is rotated as it stands, no grinding action would take place, as the vessel and its contents would rotate as a whole. To bring about a grinding or crushing action, a drag must be placed on the central steel cone, which retardation is in turn conveyed, at least in part, to the steel balls. It is therefore necessary

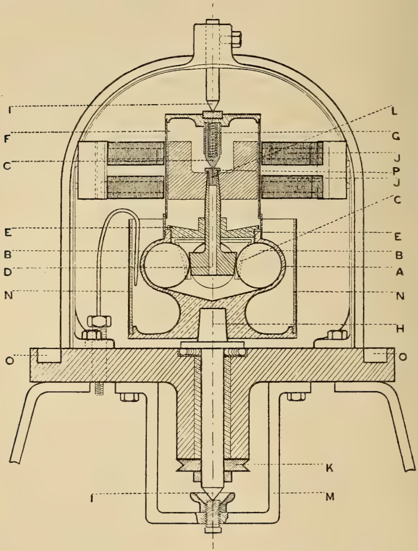


Fig. 1.—Apparatus for disintegrating bacterial or other cells. Type with electromagnetic control.

either to let the central steel cone remain at rest, or for it to rotate at a speed less than that of the steel balls. This has been effected in the type of machine now described (fig. 1), by mounting on the top end of the steel cone a bar of soft iron L, which is slightly less in length than the diameter of the covering cylinder. On each side of the machine, but near to the outside of the covering cylinder F, a pair of electromagnets are mounted, with their poles in such a position that the iron bar on top of the steel cone is attracted by them. In actual practice it is found convenient and more efficient to let this electromagnet be a circular one, so that the magnetic circuit is closed, with a pole on each side, in such a position that it will attract the central iron armature. A suitable current of electricity is then passed through the winding of the electromagnets, so that the iron armature is kept in one position. It follows that, on rotating the containing vessel, while the armature is held by the electromagnets, a drag is put on the central steel cone, which in turn is communicated to the steel balls, and the grinding action occurs in the manner indicated.

To ensure that the bacteria are brought under the influence of the grinding mechanism, the speed of rotation should be at least 1500 revolutions per minute. The bacteria are placed in the pot in a semi-fluid condition or as an emulsion. Centrifugal action then ensues, so that they are almost immediately brought under the grinding action of the balls. As the grinding action is one which takes place largely as the result of the rotation of the balls in an exactly fitting race the amount of friction is almost negligible; and further as the pressure that is put on the balls consists of a direct thrust by the steel cone, there is every opportunity for them to slip should any additional friction be introduced, or if for any reason any added load be put on the grinding mechanism.

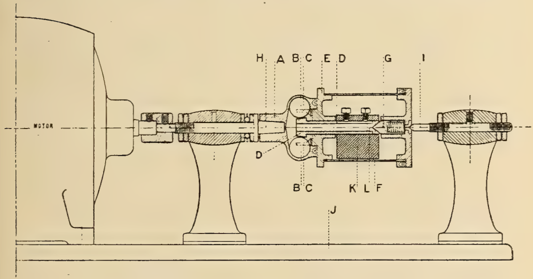


Fig. 2.—Apparatus for disintegrating bacterial or other cells. Type with gravitational control.

To ensure that the running is perfectly true, the whole appliance is mounted between centres I, I, which are adjustable for wear.

Cooling may conveniently be effected by means of liquid carbonic acid. This may be obtained commercially, ready compressed in a steel cylinder, from which the gas may be allowed to escape and impinge on to the side of the metal pot. By varying the rate of escape of the gas the temperature may be controlled at will. An alternative method is to have another containing cylinder N outside the vessel in which the balls rotate, and to pack the space between this metal cylinder and the metal pot with a mixture of ice and salt or any other convenient freezing mixture.

If pathogenic organisms are being dealt with the whole machine may be covered with a glass bell-jar. Around the base-plate a groove O is cut, and in this any fluid bactericidal agent may be put, the glass jar being then placed over the whole, thus effectually preventing the possibility of the escape of any part of the contents of the pot.

The introduction of the material to be ground into the containing vessel is effected by unscrewing the top cylinder F and taking out the small plug P at the top of the steel cone. The spindle of the central steel cone is hollow, so that the emulsified bacteria may be introduced into the vessel by means of a pipette through this opening without disturbing the balls or any other part of the apparatus. On completion of the grinding action, owing to the cell contents of the bacteria having been expressed, the material is much more fluid than when introduced; and, therefore when the machine stops, it all sinks to the depressed centre of the containing vessel, and may be pipetted off again without further trouble. The introduction into, and removal of the material from, the metal pot without disturbance of any of the parts is a matter of considerable moment when dealing with pathogenic organisms. This arrangement also enables the ground material to be removed from the containing vessel immediately the grinding action ceases. As a further precaution the lid of the pot E is recessed towards its centre as shown: it is therefore possible to fill the hollow top of the lid with a bactericidal agent; immediately the machine is started the speed of rotation ensures that this fluid is thrown outwards by centrifugal action, and completely seals the two screw-joints where the lid of the pot and the outer cylindrical cover meet. As a point in the construction it is necessary that the side wall of the top cylindrical cover should be made of vulcanite or some diamagnetic material, as otherwise the necessary magnetic pull on the armature inside would not be effected.

A simplified form of the appliance is one in which the whole apparatus is mounted horizontally, and the pull is put on the central steel cone not through the agency of an electromagnet, but by means of gravity (fig. 2). The whole arrangement is exactly similar to that previously described, except that the pot, with its balls, steel cone, and outer cover, is mounted horizontally between centres.

On the spindle of the central steel cone D a semi-cylindrical mass of iron or lead K is fixed, the weight of which has to be calculated for and proportioned to the drag on the central cone, but must be such that when the whole apparatus is rotated the weight is sufficient to keep the central cone at rest. In practice this modification has proved to be simpler than the original model, the only objection being that it is not quite so easy to cover the whole apparatus with an outside bell-jar as in the first described type.

The following experiments were carried out with the apparatus described :—

## YEAST.

In order to ascertain the efficiency of the grinding under different conditions, a series of experiments was performed with yeast. The yeast cells being large, and their cell membranes being readily recognisable after the cells have been crushed, it was possible to make an actual count of the crushed and uncrushed cells after grinding. Fresh German yeast was used and this was made into a smooth stiff cream with water for the purpose of grinding. Films were made on glass slides at various stages of the grinds; these were stained with Löffler's methylene blue, and the counts made. Controls of the unground yeast showed that practically all the cells were intact.

The results obtained were as follows :—

I. Amount = 6 c.c. yeast cream. Speed = 700—750 revolutions per minute.

(*a*) After 20 minutes' grind. Crushed cells = 597, uncrushed cells = 87. Percentage of crushed cells = 87.

(*b*) The same ground for a further period of 20 minutes. Crushed cells = 646, uncrushed cells = 32. Percentage of crushed cells = 96.

II. Similar to I. Total period of grind = 20 minutes. Examined at intervals of 5 minutes.

(*a*) After 5 minutes' grind. Crushed cells = 63, uncrushed cells = 226. Percentage of crushed cells = 21·4.

(*b*) After 10 minutes' grind. Crushed cells = 284, uncrushed cells = 343. Percentage of crushed cells = 45.

(*c*) After 15 minutes' grind. Crushed cells = 412, uncrushed cells = 113. Percentage of crushed cells = 78.

(*d*) After 20 minutes' grind. Crushed cells = 694, uncrushed cells = 59. Percentage of crushed cells = 92.

III. Amount = 12 c.c. of yeast cream. Speed 700—750 revolutions per minute. Total period of grind = 30 minutes. Examined at intervals of 10 minutes.

(*a*) After 10 minutes' grind. Crushed cells = 380, uncrushed cells = 326. Percentage of crushed cells = 53·8.

(*b*) After 20 minutes' grind. Crushed cells = 297, uncrushed cells = 195. Percentage of crushed cells = 60.

(*c*) After 30 minutes' grind. Crushed cells = 473, uncrushed cells = 109. Percentage of crushed cells = 81.

IV. Amount = 3 c.c. of yeast cream. Speed 700—750 revolutions per minute.

(*a*) After 10 minutes' grind. Crushed cells = 216, uncrushed cells = 54. Percentage of crushed cells = 80.

(*b*) After 20 minutes' grind. Crushed cells = 192, uncrushed cells = 32. Percentage of crushed cells = 85.

(*c*) After 30 minutes' grind. Crushed cells = 210, uncrushed cells = 40. Percentage of crushed cells = 84.

V. Amount = 18 c.c. of yeast cream. Speed 700—750 revolutions per minute.

(*a*) After 10 minutes' grind. Crushed cells = 46, uncrushed cells = 122. Percentage of crushed cells = 27.

(*b*) After 20 minutes' grind. Crushed cells = 127, uncrushed cells = 98. Percentage of crushed cells = 56.

(*c*) After 30 minutes' grind. Crushed cells = 154, uncrushed cells = 65. Percentage of crushed cells = 77.

VI. Amount = 6 c.c. of yeast cream. Speed 500 revolutions per minute.

After 20 minutes' grind. Crushed cells = 219, uncrushed cells = 53. Percentage of crushed cells = 80·5.

VII. Amount = 12 c.c. of yeast cream. Speed about 500 revolutions per minute.

After 20 minutes' grind. Crushed cells = 178, uncrushed cells = 79. Percentage of crushed cells = 69.

VIII. Amount = 12 c.c. of yeast cream. Speed 2000 revolutions per minute.

After 20 minutes' grind. Crushed cells = 141, uncrushed cells = 59. Percentage of crushed cells = 70·5.

IX. Amount = 12 c.c. of yeast cream. Speed 360 revolutions per minute.

After 20 minutes' grind. Crushed cells = 125, uncrushed cells = 189. Percentage of crushed cells = 39·5.

The machine did not run well at this low speed.

X. Amount = 12 c.c. of yeast cream. Speed 1400 revolutions per minute.

After 20 minutes' grind. Crushed cells = 134, uncrushed cells = 75. Percentage of crushed cells = 64.

XI. Amount = 6 c.c. of yeast cream. Speed 828 revolutions per minute.

After 20 minutes' grind. Crushed cells = 85, uncrushed cells = 18. Percentage of crushed cells = 82·5.

XII. Amount = 18 c.c. of yeast cream. Speed 750—800 revolutions per minute.

After 20 minutes' grind. Crushed cells = 233, uncrushed cells = 100. Percentage of crushed cells = 70.

XIII. Amount = 6 c.c. of yeast cream. Speed 700—750 revolutions per minute.

After 20 minutes' grind. Crushed cells = 188, uncrushed cells = 68. Percentage of crushed cells 73·5.

In Plate 1, *a* is a photo-micrograph of unground yeast, *b* and *c* the same after grinding for 15 and 30 minutes respectively.

## BACTERIA.

A direct microscopical count in the case of bacteria is impossible, and the results of grinding were therefore estimated—

(1) By a general microscopical survey of the specimens,

(2) By plating and ascertaining the number of surviving micro-organisms.

I. Cream of *Bacillus coli*. Amount = 3 c.c.

(*a*) Speed 700 revolutions per minute. Result: a good grind in 1 hour.

(*b*) Speed 1400 revolutions per minute. Result: almost complete grind in 1 hr.

(*c*) Speed 2400 revolutions per minute. Result: in ¼ hour, good grind—nearly as good as in 1 hour at 700 revolutions; in ½ hour almost complete grind.

II. Cream of *B. coli*. Amount = 3 c.c. Speed 1700 revolutions per minute. Number of living organisms estimated by means of agar plates.

Colonies on the Plates.

| Controls before grinding. | After grinding. | | | |
|---|---|---|---|---|
| | 15 minutes. | 30 minutes. | 45 minutes. | 60 minutes. |
| 1/100,000 dil.<br>0·1 c.c.<br>405 colonies | 1/100,000 dil.<br>0·1 c.c.<br>77 colonies | 1/10,000 dil.<br>0·1 c.c.<br>138 colonies | 1/10,000 dil.<br>0·1 c.c.<br>134 colonies | 1/1000 dil.<br>0·1 c.c.<br>86 colonies |
| 1/100,000 dil.<br>0·5 c.c.<br>1978 colonies | 1/100,000 dil.<br>0·5 c.c.<br>342 colonies | 1/10,000 dil.<br>0·5 c.c.<br>678 colonies | 1/10,000 dil.<br>0·5 c.c.<br>468 colonies | 1/1000 dil.<br>0·5 c.c.<br>314 colonies |
| 1/100,000 dil.<br>1·0 c.c.<br>uncountable | 1/100,000 dil.<br>1·0 c.c.<br>576 colonies | 1/10,000 dil.<br>1·0 c.c.<br>1280 colonies | 1/10,000 dil.<br>1·0 c.c.<br>1024 colonies | 1/1000 dil.<br>1·0 c.c.<br>640 colonies |

Average per Unit Volume.

| Control before grinding. | After grinding. | | | |
|---|---|---|---|---|
| | 15 minutes. | 30 minutes. | 45 minutes. | 60 minutes. |
| 400,300,000 | 67,700,000 | 13,053,000 | 11,000,000 | 709,300 |

With other bacilli, *e.g.*, *B. megaterium*, *B. mycoides*, *B. subtilis*, and *B. typhosus*, equally good results were obtained. In Plate 1, *d* is a photomicrograph of unground *B. mycoides*, *e* and *f* the same after grinding for 15 and 30 minutes respectively.

III. Cream of *Micrococcus pyogenes aureus.* Amount = 3 c.c. Speed 1700 revolutions per minute. Number of living organisms estimated by means of agar plates.

Colonies on the Plates.

| Controls before grinding. | After grinding. | | | |
|---|---|---|---|---|
| | 15 minutes. | 30 minutes. | 45 minutes. | 60 minutes. |
| 1/1,000,000 dil.<br>0·1 c.c.<br>2 colonies | 1/1,000,000 dil.<br>0·1 c.c.<br>1 colony | 1/100,000 dil.<br>0·1 c.c.<br>13 colonies | 1/100,000 dil.<br>0·1 c.c.<br>14 colonies | 1/10,000 dil.<br>0·1 c.c.<br>nil |
| 1/1,000,000 dil.<br>0·5 c.c.<br>42 colonies | 1/1,000,000 dil.<br>0·5 c.c.<br>12 colonies | 1/100,000 dil.<br>0·5 c.c.<br>33 colonies | 1/100,000 dil.<br>0·5 c.c.<br>27 colonies | 1/10,000 dil.<br>0·5 c.c.<br>14 colonies |
| 1/1,000,000 dil.<br>1·0 c.c.<br>145 colonies | 1/1,000,000 dil.<br>1·0 c.c.<br>72 colonies | 1/100,000 dil.<br>1·0 c.c.<br>41 colonies | 1/100,000 dil.<br>1·0 c.c.<br>24 colonies | 1/10,000 dil.<br>1·0 c.c.<br>27 colonies |

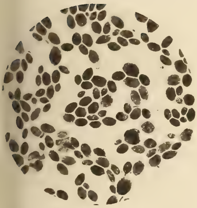
Average per Unit Volume.

| Control before grinding. | After grinding. | | | |
|---|---|---|---|---|
| | 15 minutes. | 30 minutes. | 45 minutes. | 60 minutes. |
| 83,000,000 | 35,300,000 | 7,900,000 | 7,270,000 | 275,000 |

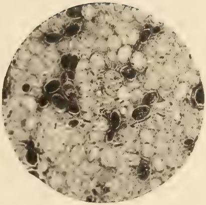
In Plate 1, *g* is a photomicrograph of unground *M. pyogenes aureus*, *h* and *i* the same after grinding for 15 and 30 minutes respectively.

## CONCLUSION.

We believe that the results of these experiments show that the apparatus here described does efficiently disintegrate bacterial cells. The apparatus is simple to manipulate, and, moreover, its design provides absolutely against the escape of any of the contents in the process of grinding, a consideration of great moment when dealing with pathogenic micro-organisms.



A



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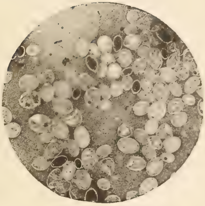

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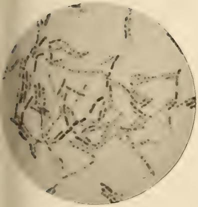

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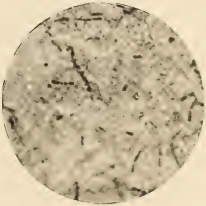

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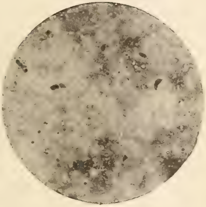

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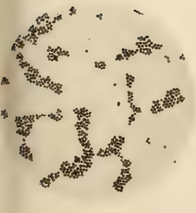
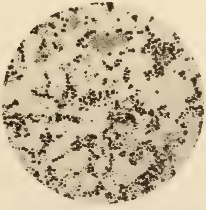
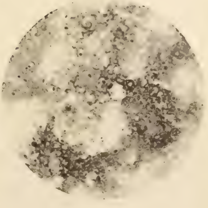


G



H



I

# *Motor Localisation in the Brain of the Gibbon, correlated with a Histological Examination.*[*]

By F. W. Mott, Edgar Schuster, and C. S. Sherrington.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received March 28,—Read May 4, 1911.)

Motor localisation in the Gibbon has not been hitherto determined experimentally, probably owing to the difficulty of obtaining a suitable animal. It appeared to be desirable, therefore, to see whether the habits and mode of life of this animal could be correlated with an increased development of the motor cortex. One of us (F. W. M.) had some years ago, by a comparative study of the convolutional pattern of the brains of Lemurs and Apes, made the following deduction:[†] "The remarkable use this animal makes of its arms and hands can be correlated with a remarkable expansion of the cortex in the precentral region, as shown by the development of a broad gyrus extending from the middle of the precentral region to form the second frontal convolution. Now if we turn to the Ape's brain (Macacus), and see what the effect of this development would be, we observe that it would push forwards and downwards that portion of the cortex which on stimulation gives rise to movement of the head and eyes, particularly that which gives rise to eye movements, etc." Figures were shown to indicate that the sulcus arcuatus would be pushed down to join the sulcus rectus. The following experiments by stimulation, correlated with a complete histological examination of the cortex in front of the central sulcus, have confirmed this deduction.

The animal used for the experiments was a male and black in colour; it was remarkably agile; when standing or running on the ground it maintained almost an erect posture, using its long arms to balance itself very much as a man would walk on a tight rope with a balancing-pole. It was kept for some days before the experiment in the animal room of the Physiological Laboratory, Liverpool, and it was frequently heard to utter vocal sounds of very varying pitch and quality. Thus it could imitate the shrill high-pitched whistles of the guinea-pig and the relatively low-pitched bark of the dog. A short account of the larynx of this animal will be made the subject of a future publication.

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[*] A portion of the expense of this research has been defrayed by a Government Grant from the Royal Society.

[†] "On the Physiological Significance of the Convolutional Pattern in the Primates," 'Brit. Med. Journ.,' 1906.

## Details of the Experiments.

The animal was anæsthetised with chloroform and ether, and a light degree of anæsthesia maintained after the brain had been exposed.

The accompanying protocol describes the results obtained, and fig. 1 L and R indicate the points of stimulation.

### Protocol of Experiments.

### *Left Hemisphere.*

*Unipolar stimulation*: diffuse electrode on R. foot; small electrode (ball-pointed, ball about 0·5 mm. diameter); stimulus in Kronecker units (K.U.):—

500 K.U.—

1. Movements of nostril.
2. Retraction of lip, opening of jaw.
3. Turning of head to opposite side.
4. Extension of elbow.
5. Ditto and movement of thumb.

600 K.U. (large electrode with ring loop for application, 4 mm. in diameter)—

6. Flexion of elbow, some retraction of shoulder.
7. Closure of eyelids.
8. Inward rotation of wrist, reaching forward movement from shoulder.

8 (again). Drawing-back movement.

6 (again). Flexion of elbow, drawing-to of shoulder.

8 (again). Drop (flexion) of wrist.

9. Extension of shoulder.
10. Extension of shoulder, accompanied by abduction of wrist, extension of fingers, with a little abduction of thumb (also relaxation of biceps).
11. Elevation of shoulder.
12. Slight flexion of knee.

*Bipolar stimulation* (stimulus value in centimetres):—

9 cm.—

13. Wrinkling of forehead.
14. Closure of lower and upper eyelids.
15. Forehead and nostril.

*Unipolar stimulation:* diffuse electrode on L. foot; loop electrode as before:—

600 K.U.—

16. Flexion of hip.
17. Flexion of knee and extension of toes and hallux (succeeded by flexion).

17 (again). Flexion of knee, extension of ankle and toes, going back into flexion.

17 (again). Flexion of hip and knee, extension of foot and toes.

18. Slight flexion of toes (without hallux), extension of ankle with some opening (*i.e.* separation) of toes.

*Bipolar stimulation* as before:—

18. (again). Slight contraction of toes.

*Unipolar stimulation* with fine ball-pointed electrode:—

17 (again). Extension of hip and knee, abduction of leg.

17 (again). Abduction.

19. Extension of foot (very slight).

20. Distinct flexion of hip and knee, flexion of toes.
20 (again). Flexion of knee, flexion of hip.
21. Mouth.
22. Retraction of tongue.
22 (repeated). Same results. ($\frac{1}{2}$ cm. of cortex for tongue.)

1000 K.U.—
23. Eyeballs turned inwards and downwards.
24. Upward movement of eyeball.
23 (again). Eyeballs turned downwards and slightly inwards.
23 (repeated). Same results.

1250 K.U.—
Eyes. No result.

*Bipolar stimulation* :—

9 cm. No result.
8 cm. Mouth moves.

*Unipolar stimulation* as before :—

1250 K.U.—
22*a*. Movement of tongue (protrusion of opposite side).
22*b* (at lowest point). Movement of tip of tongue.
25. Here a very slight movement of tongue tip was obtained from just behind inferior extremity of central fissure, tip of tongue deviated to opposite side (but see below).

*Unipolar stimulation* : small electrode :—
800 K.U.—
22*a*. In front of fissure, deviations of tongue as before.
25. And various other points behind fissure, nothing.

1250 K.U.—
22*a*–*b*. Well-marked protrusion and deviation to opposite side.
22*a*–*b* (again). Protrusion, obtained repeatedly.
22*a*–*b* (again). Retraction.
25. Nothing.
25 (again). Nothing (repeated).
(Results obtained above from 25 with large electrode attributed to diffusion.)

### *Right Hemisphere.*

*Unipolar stimulation* : coarser electrode :—
800 K.U.—
1. Extension of wrist, opening of fingers.
2. Extension of elbow and wrist, flexion of fingers.
3. Flexion of fingers, chiefly index, abduction of thumb.
4. Movements of wrist, tendency to pronation.
5. Extension of fingers, hallux, wrist; some abduction and tendency to pronation.
6. Eye-movements, outward and upward.

900 K.U.—
7. Extension of wrist.

1000 K.U.—
3 (again). Flexion of fingers and wrist (clenching of hand).

(*Interval of* 20 *minutes ; stimulation then resumed.*)

As before, but with fine electrode :—

900 K.U.—

8. Primary eversion of foot, followed by inversion ; movements of hip and knee.
8 (again). Slight eversion, then inversion.
9. Slight flexion of hip and knee, movements of trunk (pelvis raised).
8 (again). Movements of trunk, flexion of hip and knee, dorsal flexion of foot.
8 (again). Extension of foot.
9 (again). Marked extension of foot and extension of knee.

*Left Hemisphere.*

1000 K.U.—

19*a*. Dorsal flexion of (right) foot, flexion of (right) hip and knee (walking movements).
19*b*. As before ; more definite.

*Calcarine Region. (Both Hemispheres.)*

*Bipolar stimulation :* distance between points widened to 6 mm. :—

8 cm.—

1. Left hemisphere, just above polar end of calcarine ; slight movement of eyeball upwards and to left.
2 (repeated). Movement of eyeball upwards and a little inwards.
3. Right hemisphere, corresponding point to 1 ; movement of eyeball over to left in wavering manner.

6 cm.—

4. Right hemisphere, mesial surface of pole ; movement of eyeball over to left, and somewhat downwards, dilation.
5. Right hemisphere, outer surface (polar region) ; same result.
6. Left hemisphere, similar point to 5 ; eyes move to right.
7. Left hemisphere, at anterior extremity of external calcarine ; same result.

*Larynx.*

*Left Hemisphere.—Bipolar stimulation :* wide electrodes :—

6 cm.—

26. Adduction of chords.
26 (repeated). Same results.
26 (again). Adduction of both chords, but chiefly same side.

*Bipolar stimulation* (C. S. S. stimulating) :—

5 cm.—

26. Slight adduction.
26*a*. Same as 26.

The stimulation of the calcarine region of the occipital lobe was not performed until the motor area had been mapped out, consequently the cortex may not have been in such a favourable condition for excitation. Unipolar excitation gave no definite results; the stimulation so given may not have been diffuse enough. Bipolar excitation invariably produced deviation of the eyes away from the hemisphere stimulated when one pole was placed above and the other below the calcarine fissure; the regions stimulated extended from the mesial surface of the pole of the occipital lobe along the external surface to the anterior extremity. The electrodes placed elsewhere on the occipital lobe gave no movements. It may therefore be inferred that owing to the infolding of the cortex to form the fissure stimulation of this region by bipolar excitation extended to a sufficient number of motor neurones, or that it is in this region indicated in fig. 1 R by area 28 that the optic radiations terminate in greater numbers than elsewhere in the occipital lobe.

Again, it is probable that unilateral stimulation was inefficient in the production of adduction of the vocal chord, because this experiment was the last performed. Definite movements were obtained for a short time, however, by bipolar stimulation of the region 26 indicated; later on, however, the same strength of stimulus failed to give any response, and the animal was killed.

It is of interest to note that unipolar stimulation gave no result when applied to the ascending parietal convolution; this fact, as we shall see, accords completely with the histological observations.

## HISTOLOGICAL OBSERVATIONS.

At the close of the experiments, after the animal had been killed, the brain was hardened *in situ* by an injection of formalin solution through the carotid artery. It was thought that in this way the structure of the cells would be best preserved. Subsequent examination showed that this anticipation was not realised, for the preservation was not sufficiently good to make a complete survey of the cell lamination of the whole brain profitable. It was, however, quite adequate for the purpose of determining the extent of the principal areas in the lateral and mesial surfaces of the frontal lobe. For this purpose the brain was divided into blocks, arranged in such a way as to avoid, as far as possible, the necessity of cutting any part of the cortex obliquely or tangentially, and the planes of section were plotted carefully on outline drawings of the surface of the hemispheres. After the blocks had also been drawn, they were embedded in paraffin in the usual way, and cut into sections parallel to their faces. The sections were stained with polychrome methylene blue.

Both hemispheres were examined, but the results have been mapped only on the drawings of the right hemisphere (figs. 2 and 3). Since the types of cortex here dealt with have been often and fully described and figured, and since their structure in this case presents apparently no unusual features, special descriptions or drawings have not been given.

Figs. 2 and 3 show the distribution of two quite distinct types of cortex in the lateral surface of the Gibbon's brain. That portion which is marked in the diagram with a number of large and small dots is covered by a type of cortex characterised by the absence of a distinct layer of "granules" or "stellate cells," and thus corresponding to Campbell's* precentral and intermediate precentral types, or to Brodmann's† types 4 and 6. The size of the dots shows roughly the relative size of the largest cells in the ganglionic layer or inner layer of large pyramids. The largest of these dots indicate the presence of cells which may safely be called giant pyramids or Betz

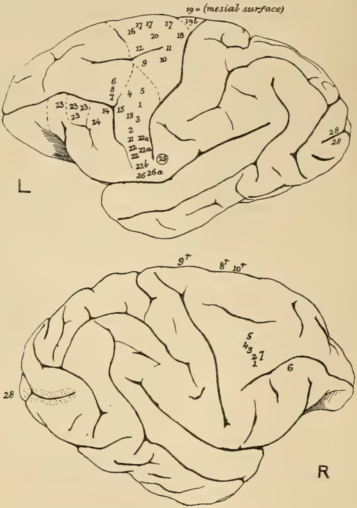
19 a (mesial surface)
16
17
17
17
19b
20
18
12
11
9
10
6
8
7
4
5
23
23
23
14
15
1
23
24
13
3
2
21
22a
22
22a
22
25
22b
26
26a
28
28
L
9†
8†
10†
5
4
3
2
7
1
6
28
R

FIG. 1.

L, Lateral Surface of Right Hemisphere; R, Mesial Surface of Right Hemisphere.

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* Campbell, 'Histological Studies on the Localisation of Cerebral Function,' Cambridge, 1905.

† Brodmann, "Beiträge zur histologischen Lokalisation der Grosshirnrinde," III, 'Journal für Psychologie und Neurologie,' 1905, bd. 4, heft 5—6.

cells; their position thus marks the extent of Campbell's precentral or motor area, or of Brodmann's type 4. The extent of the intermediate precentral cortex of the former, or type 6 of the latter, is shown by the smaller dots. The Betz cells are most numerous, largest, and cover a wider zone on the mesial surface of the hemisphere above the sulcus cinguli and on the lateral surface in the neighbourhood of the supero-mesial border (fig. 3). On the lateral surface, below the level of the sulcus precentralis superior (*prs.*), they are confined to the anterior wall of the sulcus centralis and to a narrow strip of the ascending frontal convolution lying immediately in front of that fissure.

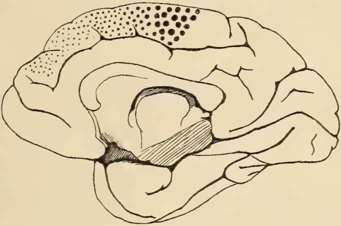

FIG. 2.

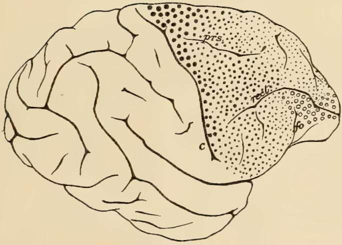

FIG. 3.

*prs.*, Sulcus precentralis superior. *rect.*, Sulcus rectus. *fo.*, Sulcus fronto-orbitalis. *c.*, Sulcus centralis.

The black dots in the above figures indicate the area covered by the precentral (motor) and intermediate precentral types of cortex; the circles the granular frontal type of cortex.

It will be seen on comparing these figures with Campbell's diagrams of the brains of the Orang and Chimpanzee, that the distribution of the Betz cells is very similar in all three cases. The Gibbon presents perhaps a slightly closer resemblance to the Orang in this respect than to the Chimpanzee.

It is the distribution of the intermediate precentral area which forms the most characteristic feature of the Gibbon's brain. The great forward extension of this area distinguishes it in a very striking way from the Orang and Chimpanzee, on the one hand, and Cercopithecus and the Baboon on the other. This extension is most marked in the region which may be described as the middle frontal convolution, namely, that portion of the lateral surface which lies between the sulcus precentralis superior (*prs.*) above, and the sulcus rectus (*rect.*) below. The area occupied by the granular frontal cortex (Campbell's frontal cortex and Brodmann's type 9) becomes in this way very much restricted, and above the sulcus rectus it occupies only the very small space in the neighbourhood of the frontal pole indicated in fig. 3 by small circles. Below that fissure the layer of granules or stellate cells is well developed in nearly the whole region lying in front of the fronto-orbital sulcus (*fo.*).

Probably as a result of the great development of the intermediate precentral area the sulcus arcuatus, the upper limit of which in Cercopithecus and the Baboon arches round the posterior end of the sulcus rectus, and lies just within or actually forms a boundary to this area, has been pushed downwards to such an extent that it has become continuous with that fissure. This condition can be recognised most clearly in the left hemisphere, where the sulcus rectus has posteriorly a well developed downwardly directed limb, which is clearly the homologue of the lower portion of the sulcus arcuatus; in the right hemisphere it is very difficult to recognise the latter at all.

Another point worthy of attention is that in the cortex of the posterior part of the middle frontal gyrus the large cells of the ganglionic layer, or inner layer of large pyramids, are somewhat larger than in the region lying above the anterior end of the sulcus precentralis superior, or below the sulcus rectus, but are not nearly so large as those which have previously been referred to as unquestionable giant pyramids.

# *Experiments on the Restoration of Paralysed Muscles by means of Nerve Anastomosis.**

By Robert Kennedy, M.A., M.D., D.Sc.

(Communicated by Prof. J. G. McKendrick, F.R.S. Received April 3,—Read June 1, 1911.)

(Abstract.)

Restoration of voluntary co-ordinated movements after "nerve crossing," first demonstrated by Flourens, has since been from time to time the subject of investigation. The conclusions of Flourens have been confirmed by Rawa, Stefani, Howell and Huber, Langley, and myself. A practical application in surgery was first suggested by Létiévant, and within the past 12 years considerable development has taken place in this direction.

During the past two years I have performed about 30 experiments on monkeys and dogs in order to investigate several points from the physiological standpoint. These experiments fall naturally into three groups. The first deals with the methods of cross union or anastomosis between the peripheral segment of a divided facial nerve and a suitable motor nerve in the neighbourhood. The second series of experiments deals with anastomosis in the fore limb of dogs, in order to investigate some aspects of the question not overtaken by previous work on this part of the subject. The third series deals with the brachial plexus, its functions, and the methods of anastomosis applied to it.

The present communication is confined to an account of experiments with the facial nerve, of which there have been 10 performed. Of these 10, 6 were primary anastomosis and 4 secondary anastomosis, that is to say, in 6 the facial was cut, and its peripheral segment immediately anastomosed with the central segment of the substitute nerve, while in 4 the facial was cut, and left unattached for a period, precautions to prevent spontaneous reunion being taken, and then at the end of that period re-exposed, and united to the substitute nerve.

### *Primary Anastomosis.*

In the primary anastomoses, two were in monkeys and four in dogs. Of the two monkeys, in one the facial was cut and attached to the side of the spinal accessory, and in the other it was attached to the side of the hypoglossal nerve. Voluntary dissociated movements of the face commenced

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* The expense of this research has been defrayed by a Government Grant from the Royal Society.

to return in the former in 58 days, and in the latter in 42 days, and each of the animals had complete voluntary closure of the eye at about 100 days.

Of the four dogs, in two the spinal accessory was the substitute nerve, and in two the hypoglossal. Of the two spino-facial anastomoses, one was an end to side, and one an end to end, and the same variation was practised with the two hypoglosso-facial anastomoses.

The two spino-facial anastomoses commenced to recover voluntary dissociated movements of the face at 105 (end to side) and 90 (end to end) days respectively, and were almost complete as regards power to close the eye at 116 and 123 days respectively.

The two hypoglosso-facial anastomoses commenced to recover power to close the eye at 55 (end to side) and 84 (end to end) days respectively, and were very complete as regards closure of the eye at 142 and 107 days respectively.

Association movements of the face, on the normal distribution of the substitute nerve being innervated by the animal, were observed only in two of the experiments, one a spino-facial (end to side) in a monkey, and one a hypoglosso-facial (end to side) in a dog. In the latter case, every rapid movement of the tongue as in eating, licking lips, &c., was accompanied by a wink.

### *Secondary Anastomosis.*

The secondary anastomosis experiments were performed in one monkey and three dogs. The monkey had spino-facial anastomosis (end to end) performed one month after section of the facial, and commenced to recover power to close the eye by means of the orbicularis at 46 days, and there was good reflex closure of the eye at 65 days.

In the three dogs the facial nerve was cut close to the stylo-mastoid foramen and precautions taken to prevent reunion, and anastomosis performed after the lapse of one month in two of the dogs, and after 100 days in the remaining dog. Of the two in which the interval of one month had elapsed, in one, a spino-facial (end to end) anastomosis, no recovery of voluntary function had taken place at 69 days, when the animal died. In the other in which a month's interval had elapsed, a hypoglosso-facial (end to end) anastomosis, voluntary dissociated closure of the eye commenced to return at 60 days and was complete at 93 days.

In the dog in which 100 days elapsed before substitution, an end to end spino-facial anastomosis was performed, and voluntary closure of the eye commenced to return at 124 and was complete by 167 days.

In every case except two, a physiological examination was made, and proved that the recovery of movements which had taken place in the face was wholly due to impulses reaching the face *viâ* the substitute nerve. In both cases in which the examination was not made before death the animals died unexpectedly. In one of these no voluntary function had returned (dog), and in the other, in which restoration had taken place (monkey), the *post-mortem* examination showed that there had been no reunion with the central end of the facial, as the stylo-mastoid foramen was found completely obliterated by a bone plug which had been hammered into it at the operation.

As an Addendum, reports of two cases of spino-facial anastomosis are given. The first is a report twelve years after the operation, performed in a woman, and published in the 'Philosophical Transactions' in 1901, in order to show the ultimate result. The second is a report of a case of facial paralysis of three years' standing, in which spino-facial anastomosis was performed, and in which recovery commenced about three years after the operation.

The following general conclusions follow from the observations which are fully recorded in the paper :—

1. In any case of facial paralysis due to division or compression of the facial nerve, the best procedure, should spontaneous recovery fail, or be deemed impossible, is to attempt restoration of the damaged nerve.

2. Should efficient restoration of the nerve be impossible or be deemed impossible, anastomosis with the spinal accessory or hypoglossal holds out the most favourable prospects of recovery, given that the facial muscles are still recoverable from the point of view of duration of complete severance from the nutritive influence of the central nervous system.

3. Of the two substitutes, spinal accessory and hypoglossal, when the latter is used the restoration appears to commence sooner, but there does not seem to be a great difference in the ultimate result of the two substitutes, as far as the recovery of the face is concerned.

4. Of the new paralysis produced as a result of cutting the substitute nerve that which is produced when the spinal accessory is cut is much less objectionable than that produced when the hypoglossal is cut, and when the paralysis is to be left as a permanent defect, namely, when the peripheral segment of the substitute nerve is to be left unattached, the hypoglossal paralysis is not justifiable.

5. When, in consequence of the anastomosis, association movements are present in addition to voluntary co-ordinated and dissociated movements, these associated movements give no trouble and are not noticeable with ordinary movements when the spinal accessory has been used, but, if present,

may be most objectionable and noticeable with ordinary movements when the hypoglossal has been used.

6. As regards the interval during which the paralysis has lasted before anastomosis has been performed, there appears to be no difference in the date of commencing recovery and ultimate result, whether anastomosis immediately follows section of the facial, or whether one month's interval at least is allowed to elapse before the anastomosis is performed after the facial has been cut.

7. The only way to make an efficient union between two nerves is completely to cut across all the nerve fibres in both nerves; methods such as Manasse's, designed to maintain the integrity of the nerve fibres, give inefficient unions.

8. In the course of recovery of independent voluntary co-ordinated movements, the orbicularis palpebrarum is first to exhibit recovery, and usually is the muscle which recovers best, and in no case has a perfect recovery in the movements of the face been proved to take place.

9. Reunion of the facial nerve is to be preferred to restoration by means of an anastomosis, as the latter involves interference with the distribution of another nerve, and association movements are sometimes troublesome.

10. The distribution of the facial nerve is, in dogs and monkeys, limited to its own side of the face, and recoveries cannot therefore be attributed to a supply from the opposite facial.

11. The distal segment of the divided facial, except for a short period immediately following division, on being irritated gives no response in the muscles, if no connections at a subsequent date have been made with the centres, either through its own central segment or by some other path, and, conversely, the occurrence of muscular responses on irritating the peripheral segment is proof that such connections have been established.

# *A Preliminary Note on the Extrusion of Granules by Trypanosomes.*

By W. B. Fry, Captain R.A.M.C.

(Communicated by H. G. Plimmer, F.R.S. Received May 31,—Read June 15, 1911.)

During some investigations carried out in the Wellcome Tropical Research Laboratories at the Gordon College, Khartoum, a phenomenon was noticed to occur which would seem to have some bearing on the life-history of the trypanosomata.

The observations were made whilst employing the dark ground method of illumination, and certain confirmatory evidence was obtained by using a modification of Levaditi's method of silver staining.

The trypanosome infection of animals referred to in this note was that caused by a strain known in the laboratories as Type I, *T. brucei* or *pecaudi*, a strain which our later conclusions lead us almost undoubtedly to regard as a variety of *T. brucei*.

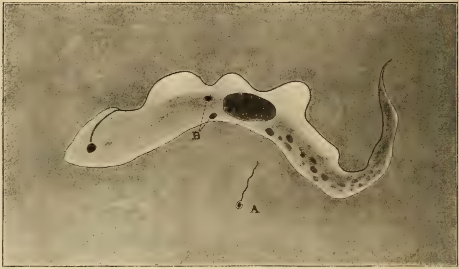
It was found that at times during the course of an infection, certain of the trypanosomes extrude from their bodies granules which are thrown off apparently with considerable force, and then appear to possess a certain motility of their own in the blood.

The phenomenon has been observed both naturally and after drug treatment; its occurrence has been studied principally in the Jerboa, an animal in which the disease runs a chronic course, and it is considered that the extrusion of the granules bears some relation to the periodic disappearance of the trypanosomes from the circulating blood.

The granule is irregularly spherical in shape and of an apparently constant size, estimated at about 0·5 $\mu$. At times a fine corkscrew-shaped filament was observed connected to these granules; this was seen sometimes immediately after extrusion; the length of the filament was estimated at four or five times the diameter of the granule. (See accompanying sketch.)

It is believed that similar granules have been observed in the circulating blood in which trypanosomes were not to be found. Further, that the same granules have been identified in certain organs, viz., the lung in dogs, during the course of an infection.

The general appearance and character of the granules were in many ways very similar to those of an extrusion granule, which was subsequently observed by Dr. Andrew Balfour in the same laboratories as occurring in the spirochætosis of fowls, accounts of which have already been published. As far as possible the general fallacies due to dark ground illumination were avoided.



Trypanosome and Granule, A, already flagellated, some time after extrusion, to show relative size. At B two granules are shown in the body of the trypanosome.

Further experiments as to the significance of this observation will be carried out later on in the year, but it is at present regarded as essentially of a vital and not of a degenerative nature.

# *The Properties of Colloidal Systems.*—II. *On Adsorption as Preliminary to Chemical Reaction.*

By W. M. Bayliss, F.R.S., Institute of Physiology, University College, London.

(Received April 7,—Read May 18, 1911.)

When a reaction takes place in a heterogeneous system certain preliminary processes occur, so that the velocity of the reaction as measured is naturally that of the slowest of all the stages, including the chemical reaction proper.

In the first place, since the reacting bodies are not uniformly distributed, one of them is compelled to travel a certain distance in order that contact with the other one may take place, *e.g.* in the case of a sheet of zinc immersed in dilute hydrochloric acid, it is necessary that the ions taking part in the reaction diffuse from the distant parts of the liquid phase in order to reach the surface of the solid phase. Hence, diffusion is the *first stage* of the reaction as a whole.

In the second place, at the interface, where the separate phases are in contact, there exists a local accumulation of energy, surface energy, as it is called. Now it has been shown by Willard Gibbs* that if a substance in solution in either phase by concentration at the surface of contact will reduce the surface energy there, such a process will of necessity take place, if it is possible. This theorem is a case of the general result of Gibbs, expressible in the following way: Increase of concentration at a surface will always occur when the potential of any form of energy at this surface can be diminished by the process. Electrical, thermal, and chemical changes are included in this statement, and not only mechanical changes such as those of surface tension. The name "adsorption" has been given to this form of condensation of bodies on the surfaces of contact between the phases of heterogeneous systems, and the name should be confined to this use. It has unfortunately been applied by some writers to any "loose" combination of an ill-defined chemical nature; thereby unnecessary confusion has been caused. There is, no doubt, evidence that the chemical configuration of the surface itself plays a part in the phenomenon,† but it is none the less essentially due to action at the surface.

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* 'Connecticut Acad. Proc.,' 1876; 'The Scientific Papers of Willard Gibbs,' 1906, vol. 1, p. 219.

† See Starling, 'Recent Advances in the Physiology of Digestion,' London, 1906, p. 40.

The mathematical expression correlating the concentration of the body in solution with the amount adsorbed by a particular surface in contact with this solution is of an exponential form, which is undoubtedly due to the manner in which the degree of diminution of surface energy is related to the amount adsorbed. We may say, then, that adsorption is, as a rule, the *second stage* in a heterogeneous reaction.

If the bodies brought into close contact by the above-mentioned surface condensation do not react with one another in the chemical sense, the whole process ends at this stage. A case of this kind is that of the condensation of aniline on the surface of mercury, investigated by Lewis.* Complete absence of chemical reaction is, however, not common. Where it occurs the rate at which it takes place varies considerably in different cases. It is important to remember, indeed, that, in agreement with the law of mass action, this velocity will be a function of the amount adsorbed, and therefore much greater than if no surface condensation had taken place. Chemical reaction is the *third and last stage* of heterogeneous reactions, and is, as a rule, the stage which conditions the rate of the process as a whole. Adsorption is rapid, and diffusion has not usually to take place through more than very short distances.†

When colloidal solutions are concerned, the "disperse" phase may consist either of ultra-microscopic particles of a solid, or of droplets of a liquid immiscible with that in which they are suspended. Moreover, the body adsorbed may be either in true solution or colloidal solution.

It will be obvious that, even if the adsorbed body does not enter into chemical combination with that upon whose surface it is condensed, nevertheless a kind of complex is produced, which may be separated from the rest of the system. Such bodies have been called "adsorption compounds," or, when both components are colloids, "colloidal complexes." In most cases it is a matter of some difficulty to show what their real nature is; their existence even is denied by many investigators. Assuming that such compounds are formed in any particular case, the velocity of the subsequent chemical reaction will be a function of the amount of the adsorption compound in existence at a given instant of time, and this again is an exponential function of the concentration of the solution of the adsorbed body. Accordingly, the form of the expression correlating the velocity of the reaction with the concentration of the reagents will also be an exponential one.

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* 'Zeit. f. physik. Chem.,' 1910, vol. 79, pp. 129—147.

† See Arrhenius, 'Immunochemistry,' p. 142, New York, 1907. Also Loevenherz, 'Zeit. f. physik. Chem.,' 1894, vol. 15, pp. 389—398.

This being so, a proof of the actual existence of such a kind of compound is of some interest. When the chemical reaction following the formation of the adsorption compound takes place very slowly, it may also be possible to obtain the latter in an isolated condition. I have, in the course of other work, met with an instance where the adsorption compound and the true chemical compound are unmistakably different bodies, both having a visible, concrete existence.

### 1. *Adsorption Compounds of Colloidal Acid with Colloidal Bases.*

If to a solution of Congo red an excess of hydrochloric acid be added, the blue free acid is precipitated; but, if the precipitate be suspended in water and dialysed, a deep blue colloidal solution is formed, as described in a previous paper.* Freshly precipitated and well-washed aluminium hydroxide is suspended in water, and a small quantity of the blue acid colloid is added. A dark blue precipitate falls, which can be washed by decantation, best with the aid of the centrifuge, and again suspended in water. It remains dark blue, and might hastily be supposed to be merely an aggregated portion of the acid colloid. That this is not so, and that the body contains also aluminium hydroxide, is shown at once by its behaviour on warming. When this is done, a red solution is rapidly formed, which, on cooling, deposits flakes of a red substance, while the solution itself becomes pale in colour. The same change occurs at room temperature, but very slowly. It is evident that we have here, in the adsorption compound formed at first, acid and base existing side by side but uncombined. On heating, chemical combination takes place with the formation of the aluminium salt of Congo red, which, like all the salts of this acid, is of a red colour. Congo red is a convenient body for the present purpose, since the salts are of a colour which is so different from that of the acid.

The precise manner in which combination is caused to take place by the action of heat does not immediately concern us; the important fact is that a body can be prepared containing acid and base uncombined.

The mode of formation of the adsorption compound is, it will be noticed, to all intents and purposes a case of the mutual precipitation of electro-positive and electro-negative colloids, in this case, aluminium hydroxide and Congo-red acid respectively.

The dry preparation of aluminium hydroxide supplied by Kahlbaum can be used, but, owing to the large size of the grains, it is not very effective. It is important that, whatever preparation be used, no free caustic alkali must be present, otherwise the red salt of the dye with this alkali is formed at once. The adsorption compound, if formed at all, only exists for an infinitesimally short time, owing to the rapidity of the chemical reaction.

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* 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 270.

In order to obtain as large a relative surface of the hydroxide as possible I have made various hydroxides in colloidal solution, prepared by dialysis of solutions of salts which are hydrolytically dissociated. Ferric chloride, aluminium acetate, zirconium and thorium nitrates have been treated in this way. With ferric hydroxide, although the result of the experiment is quite distinct, the change of colour on heating is not so obvious as with a colourless hydroxide. Aluminium hydroxide is good, but is unstable when sufficiently dialysed. The best of all those with which I have worked are the colloidal hydroxides of zirconium and of thorium, which are beautifully clear and colourless solutions. The clearness is, of course, an indication of the minute size of the suspended particles. Like all solutions prepared in the way described, they still contain, even after prolonged dialysis, traces of the original acid. If this is present in too large a proportion, no red salt is formed even on heating the adsorption compound. This fact was shown in a striking way in my first preparation of zirconium hydroxide, which had been insufficiently dialysed. In this case, although the adsorption compound was duly precipitated, it did not become red on heating. When the adsorption compound was suspended in water and subjected to further dialysis, it was noticed to be turning slightly reddish at room temperature; on boiling, the change to the red salt was immediate. The compound with thorium hydroxide seems to require heating for a longer time before combination occurs than do the others; but this may be merely owing to the presence of more acid in the particular preparation.

As is usual in the case of mutual precipitation of colloids, the readiness with which the precipitate falls depends on the rate at which the one colloid is added to the other.* If the rate is too slow, or the solutions are too dilute, the adsorption compound does not deposit but remains in suspension as a complex colloid. Moreover, a trace of a protective colloid, such as gelatin, is sufficient to prevent precipitation.

An analogous case to the one just described was met with by Van Bemmelen.† If barium hydroxide solution be added to colloidal silica, a white precipitate falls, which is found to contain both barium hydroxide and silicic acid, not in combination. On standing, barium silicate is slowly formed.

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* Freundlich, 'Kapillarchemie,' Leipzig, 1909, p. 444.

† 'Zeit. f. anorg. Chem.,' 1903, vol. 36, p. 380. Also p. 486 of the 'Gesammelte Abhandl. von J. M. van Bemmelen,' herausgegeben von Wo. Ostwald. Dresden, 1910.

The velocity of production of sulphuric acid in the method of platinum catalysis has been shown by Bodenstein and Fink[*] to be controlled by the adsorption of $SO_3$ on the surface of the platinum.

Denham[†] has made a detailed investigation of the catalytic influence of platinum on the reduction of titanic sulphate by hydrogen, and is led to the conclusion that the chief part in the reaction is played by surface condensation, in the sense of Willard Gibbs.

The work of Lewis already referred to demands further notice in the present connection. The amount of aniline condensed on the surface of mercury was found to be in agreement with that given by the formula of Gibbs deduced from thermodynamic considerations. When, however, colloids or electrolytically dissociated salts were concerned, the Gibbs formula ceased to apply. It would seem that surface energy of a kind other than that dealt with by Gibbs plays a part in these cases. In all probability the origin of this form of surface energy is to be looked for in electrical forces.

The toxic action of salts on living protoplasm,[‡] and the action of mercuric chloride as an antiseptic,[§] have been found to be proportional to the amount deposited on the surfaces of the organisms or of their constituent colloids; in other words, the exponential equation is found to apply. It is possible that chemical reactions in the strict sense of the word follow adsorption.

It has been suggested by Wo. Ostwald[||] that the taking up of oxygen by hæmoglobin is conditioned by a surface adsorption process, since the curve of percentage saturation in relation to oxygen tension is best expressed by an exponential formula. The work of Barcroft and Hill[¶] has shown that heat in definite proportion is given out when hæmoglobin combines with oxygen, so that it appears that chemical combination follows adsorption as a further stage. At the same time it should not be forgotten that the condensation of a gas is associated with the liberation of heat and in direct ratio to the amount condensed.

Findlay and Creighton[**] have shown that the solubility of certain gases in water is affected considerably by the presence of surfaces therein, and are inclined to attribute the phenomena to adsorption.

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[*] 'Zeit. f. physik. Chem.,' 1907, vol. 60, p. 43.

[†] 'Zeit. f. physik. Chem.,' 1910, vol. 72, pp. 641—695.

[‡] Wo. Ostwald and A. Dernoschek, 'Kolloid-Zeit.,' 1910, vol. 6, p. 297.

[§] Hugo Morawitz, 'Koll. chem. Beihefte,' 1910, vol. 1, pp. 317—323.

[||] 'Kolloid-Zeit.,' 1908, vol. 2, pp. 264 and 294.

[¶] 'Journ. of Physiol.,' 1909, vol. 39, p. 411.

[**] 'Trans. Chem. Soc.,' 1910, vol. 97, p. 560.

## 2. *The Part Played by Adsorption in Enzyme Action.*

In a paper published some five years ago,* I suggested the view that an adsorption compound is formed between an enzyme and its substrate preliminary to the chemical reaction properly so-called. That combination of some kind occurs is generally accepted, so that my hypothesis is to be regarded as a more definite statement as to the nature of this compound. It does not, indeed, exclude a subsequent combination of a more truly chemical nature.

The evidence in favour of the adsorption hypothesis is mainly indirect, but during the time succeeding its first publication numerous facts have come to light which confirm it in a variety of ways. Some of these facts which have been the subject of investigation by myself will be discussed in the present paper.

Two main lines of experiment have been pursued. In the first place, the process by which enzymes are removed from their solutions by substrate or inert bodies, such as charcoal or paper, will be briefly considered, and in the second place, the significance of the form of the mathematical expression correlating the concentration of the enzyme with the degree of activity will be shown.

*Adsorption of Enzymes by Surfaces.*—The work of many investigators has proved that many various substances, with which it is difficult to suppose that enzymes are capable of forming chemical compounds, are able to remove them from solution. Such bodies are charcoal, kaolin, sand, and paper. When, therefore, we find that enzymes are removed by colloidal substrates, the hypothesis naturally suggests itself that a similar process takes place, surface concentration will occur whether the adsorbed body can act further or not. I showed† that calcium caseinogenate is capable of removing from solution, in some form or other, both trypsin and diastase. Now although it is possible that there may be chemical combination in the case of trypsin and caseinogen, since chemical decomposition results from the contact, it does not seem likely that a similar state of affairs obtains in the case of diastase and caseinogen, a substrate upon which the enzyme has no action. By analogy with certain other catalytic phenomena, it seems reasonable to hold that, when chemical action results, an intermediate compound of a chemical nature has been formed, subsequent to adsorption, and that this compound afterwards breaks up, setting free the enzyme at the same time as the products of its activity.

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* 'Biochem. Journ.,' 1906, vol. 1, pp. 222—226.

† 'Biochem. Journ.,' 1906, vol. 1, p. 224.

It might be argued against the formation of chemical union of trypsin with caseinogen that when a suspension of free caseinogen (*i.e.* the free acid, not a salt) is allowed to interact with a solution of trypsin, enzyme is removed by the suspended particles, and if this complex is washed and distributed in water, no hydrolysis of the caseinogen takes place, until alkali is added. It is very possible, however, that the intermediate compound may be formed, but that it is stable in the absence of alkali.

On the other hand, Starkenstein* has described an experiment which appears to show that the adsorption and chemical compounds are bodies of a distinct nature, if indeed the latter can be said to be formed at all under the conditions in question. Amylase, obtained from the liver, was found to be quite inactive unless an electrolyte such as sodium chloride was present. Accordingly, if a dialysed preparation of the enzyme be shaken up at 40° C. with a mixture of soluble starch and ordinary starch, no formation of sugar occurs. If the mixture be filtered the soluble starch passes through the paper, leaving the rice starch behind. Now, it would be expected that, if a chemical compound were formed, the filtrate would contain it, since the enzyme would more readily combine with soluble starch than with the insoluble body, or at all events with as great readiness. On the contrary, the whole of the enzyme is found in the insoluble starch phase, as shown by the fact that addition of sodium chloride to both fractions and subsequent incubation caused the appearance of abundance of sugar in the latter but none in the filtrate.

Dietz† also has shown that the synthesis of amyl alcohol and butyric acid to the ester, as catalysed by lipase, takes place entirely in or upon the solid enzyme phase, which is insoluble in the fluid phase. If the solid be filtered off from the reacting system the filtrate undergoes no further change, although when supplied with more enzyme synthesis proceeds. The former enzyme had not lost its activity, moreover, since on adding it to more substrate, reaction went on.

It occurred to me that some light might be thrown on the question as to whether trypsin is removed from its solution by caseinogen in the form of a chemical or as an adsorption compound, by the investigation of the effect of electrolytes on the process. I have shown‡ that the absorption by filter-paper of Congo red and other electro-negative colloids is increased by the presence of neutral salts; the reason is that, both the paper and the colloid having negative charges, there is difficulty in their approximation until the charge of the paper has been neutralised or reversed by the cations of a salt in the solution. Or, to put it in another way, the surface energy of the negative paper will not be reduced by the deposition of a body with a further charge of the same sign, whereas this will happen when the two charges have opposite signs. The adsorption of electro-positive colloids by paper is retarded by neutral salts, because they reverse the difference in sign between the two bodies, making it similar, instead of opposite.

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* 'Biochem. Zeitschr.,' 1910, vol. 24, p. 218.

† 'Zeitschr. f. physiol. Chem.,' 1907, vol. 52, p. 314.

‡ 'Biochem. Journ.,' 1906, vol. 1, pp. 195—209.

The enzyme experiments were made in the following way:—Equal quantities of the various adsorbents were allowed to remain in contact with solutions of trypsin (2 per cent.) either in distilled water or in 0·018 molar solution of calcium sulphate for 18 hours. They were then filtered off, and equal amounts of each filtrate (2 c.c.) added to equal amounts (20 c.c.) of 5-per-cent. ammonium caseinogenate and then incubated at 37° C. for 4·5 hours. The relative change was measured by determinations of the electrical conductivity. The results were as follows:—

| Adsorbent. | Solvent of enzyme. | Amount of change in recip. ohms × $10^6$. |
|---|---|---|
| Caseinogen | Water | 1260 |
| ,, | Calcium sulphate | 975 |
| Soluble starch | Water | 1325 |
| ,, | Calcium sulphate | 1165 |
| Charcoal | Water | 800 |
| ,, | Calcium sulphate | 1070 |
| Filter paper | Water | 1010 |
| ,, | Calcium sulphate | 885 |
| Charcoal (2) | Water | 1015 |
| ,, | Calcium sulphate | 1050 |

In reading this table it must be remembered that a larger change means more enzyme in filtrate or *less* adsorbed.

The sign of the electric charge on all the bodies used was found to be negative, so that the result of all the experiments, with the exception of those with charcoal, corresponded to what happens when an electro-negative colloid, trypsin in this case, is adsorbed by an electro-negative surface in the presence or absence of neutral salts. In the presence of calcium sulphate more enzyme was taken up by the surface, and therefore the filtrate was less active. The opposite effect of bone charcoal is not easy to explain. It perhaps lies in the circumstance that charcoal contains a certain amount of salts (ash constituents) already, so that, even in the absence of added electrolyte, sufficient was present to ensure maximal adsorption. The slightly greater effect of the filtrate to which calcium sulphate had originally been added would then be explained by the action of the small amount of calcium sulphate contained in the 2 c.c. of filtrate on the course of the hydrolysis itself. Calcium salts are known to have a favouring effect on many enzymes. This effect may, of course, be due to the increased adsorption of enzyme by substrate in the digesting system itself. In the case of the other adsorbents, the effect of the small amount of calcium salt in the enzyme solution was overpowered by the opposite effect due to actual diminution of the total amount of enzyme present.

At the same time, experiments with sugar charcoal containing a minimum of electrolytes indicate that the above explanation of the anomalous behaviour of charcoal does not entirely account for the phenomena. It was found, in fact, that, although the sugar charcoal did not give quite so marked a difference as bone charcoal, yet it was in the same direction, and opposite to the effects with other adsorbents. The particular sample used (Kahlbaum's) was in an extremely fine powder, which could not be filtered off completely from the suspensions in distilled water. After the action of calcium sulphate it was aggregated to such a degree that filtration was easy.* The less adsorption in presence of the salt may therefore possibly have been due to the aggregation and consequent diminution of the active surface of the adsorbent. An experiment was made in order to see whether this aggregation by calcium sulphate could be prevented by the presence of a stable colloid, such as gelatin.

In this experiment it was found, indeed, that the addition of gelatin in such amount as to make a concentration of 0·5 per cent., which was too dilute to gelatinise at room temperature, prevented the aggregation of charcoal by calcium sulphate. But it also prevented the action of the calcium salt on the adsorption of trypsin by charcoal, since the filtrate from the preparation containing the gelatin contained more enzyme than that from the control preparation without gelatin. This effect is, in fact, similar to that observed when stable colloids are present in experiments on the adsorption of Congo red by filter paper under the influence of neutral salts.†

The results of the preceding section afford confirmatory evidence for the hypothesis of adsorption compounds between caseinogen and trypsin, as well as between trypsin and inert bodies. It will be seen that the behaviour of both is the same with respect to salts. Of course, the experiments afford no evidence for or against subsequent chemical combination.

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* It was also deprived of its electric charge, as shown by the absence of any movement in an electric field.

† 'Biochem. Journ.,' 1906, vol. 1, p. 201.

### *The Law Connecting Concentration and Activity of Enzymes.*

If the velocity of enzyme action is conditioned in any given case by the amount of adsorption which has taken place, it follows that when the relative concentration of enzyme and substrate is varied, the corresponding change in the rate of action will be an exponential function of the concentration. If, for example, the concentration of the enzyme is doubled, the velocity of the reaction will not thereby be doubled; if the velocity with concentration of enzyme $= 1$ be called $a$, that with enzyme concentration $= 2$ will not be $a \times 2$, but an expression of the form $a \times 2^{1/n}$ will be required, where $n$ is, as a rule, any number between 1 and 2.

It is not the purpose of the present paper to find a general law which shall apply to all enzymes under all conditions. The experiments to which I intend to refer were made in order to see whether it is necessary to make use of an exponential form of expression, in which it may be regarded as probable that the value of the exponent will vary from case to case. If this is so, we may reasonably conclude that an adsorption process is the controlling factor.

In all experiments whose object it is to compare the action of the same enzyme in varying concentrations, it is imperatively necessary that the times taken by each respectively to produce the same amount of change be taken as the basis of comparison. It is very usual to find that, during the course of a reaction progressing under the action of an enzyme, substances are formed which act either as accelerators or retarders as the case may be. A species of autocatalysis, positive or negative, takes place. For example, trypsin acts most rapidly in alkaline solution, but in the process of hydrolysis of proteins by its agency, amino-acids are produced which neutralise a larger and larger part of the alkali as the reaction goes on. On this account it is inadmissible in accurate work to take as basis of comparison the different amounts of change produced in equal times; the reaction is not at the same stage in both, so that the concentration of accelerator or retarder is different. If the object is merely to detect whether one of the two solutions is stronger than the other, of course the change in equal times may be compared.

My experiments have been made with trypsin and with invertase.

The trypsin experiments were done in the following way: A number of small stoppered flasks fitted with electrodes for the purpose of measuring the electrical conductivity of the contents were supplied each with 10 c.c. of 5-per-cent. ammonium caseinogenate. When warmed in the thermostat at 39° C., 2 c.c. of trypsin solution in the various dilutions required were added in turn to each and the electrical conductivity determined at intervals.

I have shown* that this property gives an accurate estimate of the amount of chemical change which takes place in a trypsin digest. The trypsin used in most experiments was a preparation obtained from Hopkin and Williams, occasionally an extract of pancreatin "Rhenania" was used. For dilution it was thought necessary to make use of a boiled trypsin solution of the same concentration as that to be diluted, since the electrolyte concentration would be more nearly equal than if water were used. From the data thus obtained curves were constructed, from which it was easy to obtain the time taken by each flask to arrive at the same stage of the reaction. In many cases it was possible to compare the times taken to reach the various steps, for example, an increase of 1400, 2000, 3000, etc., recip. megohms in conductivity. The velocities of the reactions being inversely proportional to the times taken to effect a given change, the reciprocals of the values got from the curves were calculated and multiplied by a number, usually $10^4$, in order to avoid fractions. As would be expected, it was found that, not infrequently, two or more of the members of a particular experiment would show an anomalous behaviour, easily detected on the curve and usually found to be due to incipient putrefaction, although toluene was always added at the commencement. In the tables below these values are placed in brackets. These tables are given in order to show the kind of data obtained, Tables I and II from two caseinogen experiments and Table III from one with gelatin as substrate. No object would be gained by multiplying these tables, since they serve to bring out the exponential law. The numbers under the head of trypsin concentration are merely relative to one another. The highest, called 256, is that of 2 c.c. of a 2-per-cent. solution, so that the lowest is 1/256th of this.

Table I.

| Concentration of enzyme. | Reciprocal × $10^4$ of time to effect change of | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1400. | | 2000. | | 3000. | 4000. | 5000. |
| 1 | 3·35 | D | 1·925 | | 1·164 | | |
| 2 | 5·00 | | 2·78 | | 1·518 | | |
| 4 | 7·41 | | 4·265 | | 2·36 | 1·573 | 1·178 |
| 8 | 11·58 | | 6·77 | | 3·55 | 1·954 | 1·335 |
| (16) | (51·35) | | (31·25) | | (14·3) | (7·05) | (3·39) |
| (32) | (33·33) | | (11·8) | | (2·73) | (1·22) | |
| 64 | 200·0 | C | 111·1 | B | 55·55 | 22·24 | 7·25 |
| 128 | 333·3 | | 200·0 | | 124·3 | 52·3 | 32·4 |
| 256 | 500·0 | | 400·0 | | 257·7 | 131·0 | 51·45 |

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* 'Journ. of Physiol.,' 1907, vol. 36, p. 221.

Table II.

| Concentration of enzyme. | Reciprocal × 100 of time to effect change of | |
|---|---|---|
| | 800 at initial stage. | 500 in middle of reaction. |
| 1 | 2·7 | 0·695 |
| 2 | 5·27 | 1·235 |
| 4 | 10·0 }A | 1·9 }E |
| 5 | 13·35 | 2·08 |
| 8 | 22·25 | 2·44 |

Table III.

| Concentration of enzyme. | Reciprocal $\times 10^4$ of time for change of | | |
|---|---|---|---|
| | 350. | 725. | 900. |
| 40 | 3330·0 | 445·0 | 162·0 |
| 20 | 1540·0 | 133·4 | 44·4 |
| 10 | 1000·0 | 45·9 | 15·35 |
| 4 | 250·0 | 9·52 | |
| 2 | 133·4 | 6·12 | |
| 1 | 67·0 | $<3·55$ | |

The letters with brackets refer to the similarly labelled curves of fig. 1.

It will be seen from these numbers that there is no obvious relationship between the activities of the various concentrations.

In order to find out whether the simplest form of the adsorption equation

$$x = y^{1/n},$$

where $x$ is the reciprocal of the time taken to effect a given change, and $y$ the concentration of the enzyme, will satisfy the experimental data, the most direct way is to take the logarithmic form of the equation, viz.—

$$\log x = \frac{1}{n} \log y,$$

and plot the values on logarithmic paper. If $1/n$ is constant, then the values will lie on a straight line and the tangent of the angle made by this line with the axis of abscissæ will be the value of $1/n$.*

Fig. 1 gives a few cases to illustrate how far the experimental results agree with a simple exponential law.

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* See Freundlich, 'Zeitschr. f. physik. Chem.,' 1907, Band 57, p. 391; and Ostwald, 'Lehrbuch der allgem. Chemie,' 1906, 2te Aufl., Band 2, Teil 3, p. 232.

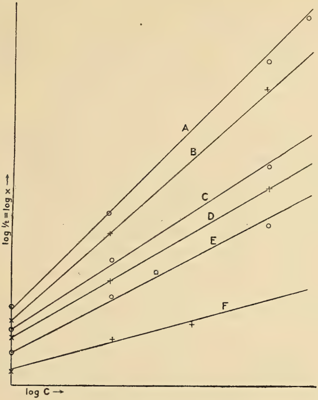

FIG. 1.

Data from Fig. 1.

| | Angle ($\theta$). | $\tan\theta$ ($= 1/n$). | $n$. |
|---|---|---|---|
| | ° | | |
| A ............... | 45 | 1 | 1 |
| B ............... | 42 | 0·9 | 1·11 |
| C ............... | 33 | 0·65 | 1·55 |
| D ............... | 30 | 0·58 | 1·72 |
| E ............... | 27·5 | 0·62 | 1·92 |
| F ............... | 15 | 0·27 | 3·7 |

The numbers show, by taking values of $n$ from different experiments, that it is fairly constant for values of enzyme concentration not very far removed from one another and for the same stage of the reaction. It tends to approximate to unity at the beginning and end of the reaction, but is usually about 1·7. Very rarely is it found to be exactly 2, as the Schütz-Borissoff law requires; although, if a particular limited region in the middle of the reaction be taken, it may have a value very near this, as shown by curve E, which represents the part of a trypsin experiment where the increase of conductivity changes from 1300 to 1800 recip. megohms. The total change being about 3500, this is therefore the middle part of the reaction.

Invertase was taken as a case where only one of the components of the system, viz. the enzyme, is in the colloidal state, the substrate, cane-sugar, being in true solution. It might be expected that the conditions would be such as to give a relationship more nearly that of a linear one.

The invertase experiments were made at a temperature of 25° C., in order that the rate of the reaction should not be too rapid. Samples were removed at intervals, mercuric nitrate added to precipitate the enzyme, excess of the reagent removed by caustic soda, filtered, made up to known volume, and finally the optical rotation determined for the mercury green line, using a three-field polarimeter by Schmidt and Haensch. The results were dealt with in a manner similar to those with trypsin and give the curve F of fig. 1. The value of $n$ for the stage of the reaction taken, viz., time taken for one-quarter inversion, is unexpectedly high, 3·7. I am unable to suggest an explanation for this wide divergence from both the linear and the square-root "laws." The separate curves of velocity of reaction were very nearly straight lines.

It seemed that it would be of interest, in view of the way in which the value of $n$ in the adsorption of Congo red by paper is affected by the presence of electrolytes, to investigate how it is altered in the case of enzyme and substrate when electrolytes are present. I have shown* that, in the former case, the value of $n$ is increased by the presence of electrolytes, so that the process approaches more nearly to a chemical one, the amount taken up being more nearly the same at different concentrations. In a chemical reaction with precipitation, of course, $n$ is infinite, since $y^{1/n}$ must be unity.

As an example from the case of Congo red and paper, $n$ was found to have the value of 1·15 in the absence of foreign electrolytes and 1·67 in the presence of 0·002 molar calcium sulphate, or 0·04 molar sodium chloride.

The corresponding experiment with trypsin was performed as follows :—

Four conductivity vessels were filled with :

A. 10-per-cent. ammonium caseinogenate 5 c.c. + $H_2O$ 5 c.c. + 5-per-cent. trypsin 2 c.c.
B. „ „ + „ + 5/3-per-cent. „ 2 c.c.
C. „ „ + 0·013 m. $CaSO_4$ 5 c.c. + 5-per-cent. trypsin 2 c.c.
D. „ „ „ + 5/3-per-cent. trypsin 2 c.c.

In thermostat at 38° C. Conductivity determined at intervals, curves drawn, and values of $n$, etc., calculated as in previous experiments. Table IV gives the data obtained.

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* 'Kolloid-Zeits.,' 1910, vol. 8, p. 4.

Table IV.

| | Time required for a change of 1200 recip. megohms in middle of curve. | Time for change of 1800 recip. megohms reckoned from beginning of action. |
|---|---|---|
| A ......... | 49 | 33 |
| B ......... | 123 | 105 |
| C ......... | 63 | 42 |
| D ......... | 97 | 100 |

It may be seen that the presence of calcium sulphate caused in certain cases or stages of the reaction an increase in the rate of the reaction, sometimes a diminution. The increase was more marked with the lower concentration of enzyme. From these figures the value of *n* comes out—

Water, from column 1, 1·2, from column 2, 1·03,
$CaSO_4$ „ 7, „ 1·3.

The effect on *n* is therefore of the same nature as in the case of a simple adsorption, viz., an increase. In other words, there is less difference between the activity of different concentrations of enzyme in the presence of calcium sulphate than in the absence of neutral salt. An investigation of the causes leading to the various numerical values at various stages of the reaction or with various concentrations of enzyme would no doubt throw light on the manner of its action.

The greater effect on the rate of the reaction in the case where the enzyme is present in lower concentration is, I think, what would be expected. When the enzyme is in excess, the additional amount adsorbed under the influence of calcium ions would probably have comparatively little effect, since it is not unlikely that the amount adsorbed without this influence is already capable of maximum hydrolytic action on the substrate.

In another experiment, in which dialysed trypsin was used, the following values of *n* were obtained :—

| | Beginning of reaction. | Middle of reaction. | End of reaction. |
|---|---|---|---|
| Water ......... | 0·94 | 1·7 | 2·48 |
| $CaSO_4$ ......... | 5·7 | 2·36 | 1·81 |

The action of electrolytes on trypsin is evidently a somewhat complex one, and requires further research.

It is held by some that the well-known fact that the presence of substrate protects an enzyme from the destructive action of a raised temperature indicates chemical combination between the two bodies. It seemed, therefore, of interest to see whether an indifferent substance like charcoal would also protect enzymes by adsorbing them. In order to make the conditions equal in comparing the activity of a preparation which had been heated in presence of charcoal with that which had been heated alone, charcoal in the same amount was added to the latter after heating and when caseinogen was added to both. It is necessary to remember that, as Hedin has suggested,* when the adsorption compound of trypsin with charcoal is brought into caseinogen solution, the latter body may remove the enzyme from the charcoal by its greater adsorption "affinity." The first experiment, in which the enzyme was heated only to 47° for an hour, showed little loss of activity, and to the same extent both with and without charcoal. Another experiment, in which the solutions were heated to 60° for 10 minutes, showed that charcoal had protected the enzyme to a certain degree. The activity of the enzyme heated in presence of charcoal was such as to cause a change of 1440 recip. megohms in two and a quarter hours, as against one of 1270 recip. megohms in the case of the enzyme heated alone.

Simple adsorption, therefore, exercises a protecting influence, and it is unnecessary to postulate chemical combination in order to explain the protective action of substrate or products.

The fact that there is an exponential relation between the concentration of an enzyme and the degree of its activity suggests that some kind of an adsorption process intervenes as a factor in the rate of the reaction. To obtain an equation for trypsin in which the value of the exponent is constant would be a matter of much difficulty, since the adsorbing surface is continuously changing as the chemical reactions split up the colloidal substrate to amino-acids, etc. Moreover, as recent work by G. C. Schmidt† has shown, the simple adsorption equation given on a previous page applies only to a particular and somewhat narrow range of concentration in various adsorption processes. In order to include wide differences, a more complex formula is required. For our purpose, it is sufficient to bear in mind that an apparently linear relationship in the initial part of a reaction, or with very low concentration of adsorbent, does not preclude adsorption. Again, Denham has pointed out‡ that the results of Frankland Armstrong§ on lactase, in which there seems to be a different law correlating concentration and action for large and small concentrations of substrate, are readily explained by the slight effect of sugar on surface tension.

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* 'Biochem. Journ.,' 1906, vol. 1, p. 481.

† 'Zeitsch. f. physik. Chem.,' 1910, vol. 74, p. 689.

‡ 'Zeitsch. f. physik. Chem.,' 1910, vol. 72, p. 690.

§ 'Roy. Soc. Proc.,' 1904, vol. 73, p. 508.

A similar view to that advocated in the preceding pages as to the nature of the combination between enzyme and substrate is described by Freundlich* as applying to the process of tanning leather. The amount of tannin taken up is conditioned by an adsorption process in the first instance. This is followed by a chemical reaction in the true sense, which takes place slowly and results in the formation of insoluble bodies. In the case of enzymes, the chemical reactions subsequent to adsorption are more rapid than in the above process.

The *temperature coefficient* of enzyme action as a whole is known to be a high one, whereas that of adsorption is, so far as investigated up to the present, a low one. In the case of Congo red and paper, I have found† that the rate at which equilibrium is attained is accelerated by rise of temperature, but that the coefficient is only 1·36 for each 10 degrees between 10° and 50° C., thus corresponding closely with that found by Brunner‡ for a diffusion process, viz., 1·5. It seems then that the effect of temperature on the rate of adsorption is merely on the rate of diffusion of the bodies concerned. In the case of Congo red and paper it is of interest to note that the actual amount adsorbed is diminished by rise of temperature, although the rate at which the smaller amount is taken up is increased.

In the case of enzyme action the chemical change, being the slowest member, is the factor governing the rate of the reaction as a whole, so that the temperature coefficient is the high one of a chemical reaction. As far as the effect of temperature on the actual amount of the adsorption of enzyme and substrate is concerned, it will be seen that, if this effect is similar to that when Congo red and paper are in question, the velocity of the reaction as a whole, since it depends on the amount of enzyme adsorbed, will be a very complex function of temperature.

## *Summary.*

The existence of an "adsorption-compound" containing acid and base uncombined chemically, and which can be isolated, is described, together with the manner of its conversion into the true chemical compound or salt.

It is shown that a similar kind of compound is formed between an enzyme and its substrate, preliminary to the particular chemical change brought about by the enzyme in question.

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* 'Kapillarchemie,' Leipzig, 1909, p. 532.

† 'Biochem. Journ.,' 1906, vol. 1, p. 187.

‡ 'Zeitsch. f. physik. Chem.,' 1904, vol. 47, p. 62.

Adsorption between enzyme and substrate as affected by the presence of neutral salts is investigated and found to follow the laws of "electrical" adsorption.

The relation between the concentration of an enzyme and its activity is shown to be expressed by an exponential formula, the value of the exponent varying considerably according to circumstances. In certain conditions it may be unity and in others the square root, but is usually between the two.

Accordingly, the view that the rate of an enzyme action at any given moment is a function of the amount of the adsorption compound of enzyme and substrate in existence at that time is to be regarded as fairly well established.

The expenses of the research were defrayed from a grant by the Government Grant Committee.

# *On the Distribution and Action of Soluble Substances in Frogs Deprived of their Circulatory Apparatus.*

By S. J. Meltzer, New York.

(Communicated by Prof. A. R. Cushny, F.R.S. Received April 11,—
Read May 18, 1911.)

(From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.)

In view of the great distributory efficiency of the cardio-vascular apparatus, no serious consideration has been given to the possibility of the existence of other modes of distribution of material in the animal body. In the following, results of experiments will be briefly presented which give unmistakable evidence of an efficient distribution of substances in cardiectomised frogs.

In these experiments the heart was exposed, ligated, and removed, and the incision closed again. Such a removal of the heart eliminates also the activity of the lymph vessels and the lymph hearts which empty their contents into veins. Injections were given into the various lymph sacs of the body and into the abdominal cavity. The results to be reported were derived from experiments made with three alkaloids presenting different types: adrenaline, strychnine, and morphine.

*Adrenaline.*—The dilating effect upon the pupil was the leading reaction. An injection of 1 c.c. of adrenaline causes in cardiectomised frogs sooner or later a dilatation of the pupil. It may appear in less than an hour (dorsal and lateral lymph sacs), or after two hours (abdominal cavity or in one leg). When injected into both legs the dilatation appears much sooner. Cutting both sciatic plexuses does not interfere with the appearance of the dilatation. The dilatation sets in even if the animal is suspended by the head. When injected into a lateral lymph sac the pupil of the corresponding side dilates first, to be followed 20 or 30 minutes later by a dilatation of the pupil of the other side.

When the frogs are kept moist and at a low temperature the pupils dilate after an injection of adrenaline even three or four days after cardiectomy, provided the eyes are not dried out. In the latter case the presence of adrenaline in the orbit is easily demonstrated by placing a fresh bulbus from a normal frog with the corneal surface inside. In this manner the pupils of several normal bulbi may become dilated by being placed in the orbit consecutively one after another.

These experiments demonstrate that adrenaline may become distributed through the entire body of a frog in the absence of the circulatory apparatus. In the case of the migration from a lateral lymph sac to the eye of the opposite side, the adrenaline has to pass through fairly solid membranes and voluminous masses. Diffusion alone will probably not accomplish it; osmosis will have to assist in the process. Gravity is not an essential factor. Movements of the animal, or "vital" activities of cells, are not parts of this peripheral mechanism of distribution.

*Strychnine.*—Frogs survive cardiectomy an hour or two, or even longer, according to the temperature at which they are kept; spontaneous and reflex movements disappear gradually. Strychnine exerts a definite influence upon the course of life after cardiectomy. When about 10 mgrm. of strychnine are injected, after a temporary insignificant depression, the animal develops in 30 or 40 minutes a definite tetanus. These animals invariably survive the controls, kept under the same condition, by an hour and longer. When a somewhat larger dose is administered, the first effect is a definite depression which may be accompanied by a semi-paretic state. Suddenly the animal asserts itself, becomes hyperæsthetic, and develops a tetanus. When a still larger dose of strychnine is injected, the essential effect is an early onset and development of an unmistakable paralysis; the latter can no longer be overcome by the hyper-excitability which ineffectively manifests itself later.

Animals in which strychnine produced an early definite depression and a paretic state are survived by the controls.

Strychnine, then, is readily distributed in the cardiectomised animals and produces there vital phenomena similar to those seen in normal frogs, that is, tetanus and paralysis. The paralysis in the cardiectomised frogs is central and is not due to fatigue. The occurrence of a violent tetanus in these animals refutes conclusively the theory of Verworn, that the paralysing action of strychnine is due to its paralysing effect upon the heart.

*Morphine.*—When about 10 or 15 mgrm. of morphine have been injected into a normal frog, no effects will be noticed until a few days later, when it may develop a tetanus. A cardiectomised frog, however, reacts to morphine in an entirely different manner. A small dose of morphine, 6 or 8 mgrm. for a medium-sized frog, will bring out a tetanus in 40 or 50 minutes. After a larger dose, the tetanus is preceded by depression and weakness. After a still larger dose, the effect is paralysis with very little evidence of hyperexcitation. In short, in cardiectomised frogs morphine affects the central nervous system very rapidly, the effects being nearly like those of strychnine, that is, tetanus with smaller doses, and paralysis with larger doses. The most plausible explanation of the surprising fact is, perhaps, this :—The central circulation receives secretions from all organs and tissues, and conveys them rapidly to all parts of the body; the action of each secretion, therefore, and of all substances taken up into the circulation, is modified by the neutralising effects of various secretions. In the absence of the circulation there are no such modifying effects to interfere with the specific action of some substances.

The experiments demonstrate that in the absence of the central circulation substances may be distributed through the body by a mechanism which in some instances may act even more promptly than the cardiac mechanism. In contradistinction to the central apparatus we may designate the distributing agent in question as a peripheral mechanism. The path of distribution employed by this mechanism can be nothing else than the tissue spaces. About 15 years ago we* insisted that these are more or less efficiently connected throughout the body, and present a unity, a system of their own. A similar peripheral mechanism, working through a similar path, is probably active in the distribution of mesolymph in animals still without a cardio-vascular apparatus. In animals possessing such an apparatus the peripheral mechanism may perhaps have the significance of a phylogenetic phenomenon.

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* Adler and Meltzer, 'Jour. of Exper. Med.,' 1, 512, 1896.

The presence of an acting peripheral mechanism in cardiectomised animals suggests the following possibilities:—

1. That the peripheral mechanism is active to some small degree in all parts of the normal body; it is, perhaps, this mechanism which favours local action of substances. 2. That this mechanism may take an active share in the process of distribution in organs which are normally deficient in circulation. The brain, for instance, has no lymphatics, and the exchange of fluid material with the blood capillaries is said to be there somewhat deficient. 3. That the peripheral mechanism gets into prominence in pathological conditions in which there is either a local or general deficiency of the cardio-vascular circulation.

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# *The Mechanism of Carbon Assimilation : Part III.*

By Francis L. Usher and J. H. Priestley.

(Communicated by Dr. M. W. Travers, F.R.S. Received April 13,—Read June 1, 1911.)

Some experiments and conclusions recorded in two papers* published in 1906 have been subjected to criticism by several investigators, and the present paper has been written with the object of presenting some new facts bearing on the problem of carbon assimilation, which incidentally support some of those conclusions. We also take this opportunity to restate the theory originally advanced, with such modifications as may be necessary, and to reply to a few of the more important objections to it which have been raised.

The observations recorded below are concerned only with the initial stages of the photosynthetic process, that is to say, with the formation of the primary photolytic products from carbon dioxide, and with the evolution of oxygen. In the papers referred to some evidence was given in support of the belief that aqueous carbon dioxide is decomposed by light under the conditions obtaining in a green leaf, the immediate products of this decomposition being hydrogen peroxide and formaldehyde; and it is easy to see that the production of these two substances would satisfactorily account both for the oxygen and the carbohydrate, which are the first visible results of the natural process. As the evidence put forward was to some extent indirect,

* 'Roy. Soc. Proc.,' B, vol. 77, p. 369 ; and vol. 78, p. 318.

wholly so in the case of hydrogen peroxide, it was thought advisable to supplement it by further experiments.

### 1. *The Products of Photolysis of Aqueous Carbon Dioxide.*

(a) *In Vitro.*—No further experiments have been made with solutions of uranium salts; either no sensitiser at all, or chlorophyll films, as described in Part II, have been employed. It has been found possible to decompose an aqueous solution of carbon dioxide without either an optical sensitiser or a reducing agent, by supplying it with energy in two different ways, viz.: (1) By bombarding it with $\alpha$- and $\beta$-rays from radium emanation and its products, and (2) by exposing it to the light emitted by a quartz mercury-vapour lamp.

The experiment with $\alpha$- and $\beta$- rays was carried out as follows:—About 200 c.c. of distilled water were saturated with carbon dioxide, and into this solution about 0·0001 c.c. of radium emanation was introduced. After four weeks the solution was tested for formaldehyde by Schryver's method.* It contained an appreciable quantity of formaldehyde, a well-marked red colour being observed when the test was applied. The greater part of the aldehyde was in a polymerised form, but no sugar was detected. Another portion of the liquid gave a yellow coloration with a solution of titanium oxide in sulphuric acid, showing the presence of hydrogen peroxide.

The recently published investigations of Kernbaum on the action of $\beta$-rays† and of ultra-violet light‡ on water, in which the author stated that hydrogen and hydrogen peroxide were simultaneously produced, suggested an examination of the action of ultra-violet light on solutions of carbon dioxide. In a preliminary experiment, a shallow glass dish containing distilled water was placed immediately beneath, and 2 to 3 cms. from, a quartz mercury-vapour lamp, and carbon dioxide was bubbled through the water while the lamp was in action. After two hours' illumination the water contained hydrogen peroxide, which was identified by the titanium sulphate reaction, as well as a small quantity of formaldehyde. A blank experiment, without carbon dioxide, was then carried out for the same length of time, but in this case formaldehyde was again detected, in addition to hydrogen peroxide. This may have been due to the presence of atmospheric carbon dioxide, but since it was possible that the formaldehyde might have been formed as a decomposition-product of dust particles from the air or the water, the experiment was repeated with greater precautions.

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* 'Roy. Soc. Proc.,' B, vol. 82, p. 226.

† 'Comptes Rendus,' 1909, vol. 148, p. 705.

‡ *Ibid.*, 1909, vol. 149, p. 273.

Two transparent quartz tubes, of about 20 c.c. capacity, were filled with the purest "conductivity" water obtainable, and the tubes were inverted in a trough of mercury and placed symmetrically near the mercury-vapour lamp. The water in one of the tubes was as nearly as possible gas-free, and a few cubic centimetres of carbon dioxide were introduced into the other. Both tubes were illuminated for about 12 hours, and the contents of each were then examined for the presence of formaldehyde and hydrogen peroxide. The solution of carbon dioxide was found to contain an easily recognisable quantity of formaldehyde, most of which was in a polymerised form, whereas none, either free or polymerised, could be detected in the water from the other tube. Traces of hydrogen peroxide were present in both. All the reagents used were carefully tested, and negative results were obtained with a solution of carbon dioxide which had not been exposed to ultra-violet light.

It appears from these experiments that ultra-violet light can effect a measurable decomposition of aqueous carbon dioxide without the intervention of an optical or chemical sensitiser, whilst under normal conditions some such agent is required;* moreover, the results furnish very strong support for the belief that both formaldehyde and hydrogen peroxide are formed in a green leaf.

A considerable number of experiments which have been carried out with chlorophyll films point to the same conclusion, and the results of four which may be regarded as typical are tabulated below:—

| *Description of Experiment.* | *Remarks.* |
|---|---|
| (i) Sealed tube containing chlorophyll painted over a layer of gelatine, made up with catalase solution, on a glass plate. Air and caustic potash present. | Both these tubes were set up at the same time, at 5 P.M., 22/4/09. At 6 P.M. (ii) showed signs of bleaching. At 12 noon, 23/4/09, (ii) was much bleached, whilst (i) was still quite green and distorted with bubbles. |
| (ii) The same as (i), but without catalase. | |
| (iii) The same as (i). | Both set up at 11.30 P.M., 24/4/09. At 11 A.M., 25/4/09, (iv) was considerably bleached and developed a very strong coloration after 5 minutes' immersion in Schiff's reagent. (iii) was still quite green, and showed the faintest coloration only after 15 minutes' immersion. |
| (iv) The same as (i), but with a solution of carbon dioxide instead of caustic potash. | |

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* Berthelot and Gaudechon ('Comptes Rendus,' 1910, vol. 150, p. 1690) obtained formaldehyde by the action of ultra-violet rays on carbon dioxide in the presence of a reducing agent. Such a reducing agent may have been present in our experiments in the form of hydrogen resulting from the decomposition of water (Kernbaum, 'Comptes Rendus,' vol. 149, p. 273, and Tian, *ibid.*, 1911, vol. 152, p. 1012). See also in this connection Stoklosa and Zdobnicjý, 'Anz. kais. Ak. Wiss. Wien,' 1910, No. 19, p. 319.

The above are given as a specimen of a large number of experiments of a similar nature, the results of which leave no doubt in our minds that the bleaching of chlorophyll in sunlight, whether carbon dioxide is present or not, is due to the formation of hydrogen peroxide. The direct oxidation of chlorophyll, in the absence of water, gives rise to a brown and scorched appearance, very different from the true bleaching observed when water is present. As regards the production of formaldehyde, the experiments are equally conclusive in showing that it is only detected by Schiff's reagent when carbon dioxide is present. Great care must be taken to remove any carbon dioxide dissolved in the films used in control experiments.

(b) *In the Plant.*—Generally the same results have been obtained with green tissues, although in this case the observations are not quite so uniformly consistent as in the film experiments, owing to the greater difficulty in controlling experimental conditions. None of these experiments are recorded, since their evidential value is certainly inferior to that of the more easily controlled extra-cellular experiments.

It may be as well to state here that, although no results have been obtained which require any substantial alteration in the theory originally advanced, the following modifications of the conclusions given in Parts I and II have been found necessary:—(1) The statement that the "catalase" enzyme is exclusively localised in chloroplasts and amyloplasts must be abandoned. It appears to have been based on a careless observation, and subsequent experiments have merely indicated a greater concentration of the enzyme in the chloroplasts, for when the green juice obtained by pounding fresh leaves in a mortar is filtered, the green residue containing the chloroplasts decomposes hydrogen peroxide vigorously, whereas the filtered juice is relatively inactive. (2) The bleaching of chlorophyll, whether in or outside of the plant, does not require the presence of carbon dioxide; there is, however, now even more reason to believe that the process is dependent on the formation of hydrogen peroxide.

The whole problem of the production of formaldehyde, both in the plant and in artificial arrangements, can be more satisfactorily dealt with in the way described and experimentally illustrated by Schryver,* and the method may be employed to yield quantitative results. All the experiments recorded in this paper in which Schiff's reagent was used to detect the aldehyde were performed before the work of Schryver was published.

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* *Loc. cit.*

### 2. *The Evolution of Oxygen.*

(a) *In Vitro.*—A method of showing the evolution of oxygen from chlorophyll films in contact with catalase, different from that previously described, has been devised by making use of Beijerinck's luminous bacteria. A pure culture of these bacteria has been observed to glow only in the presence of free oxygen. The experiment, which is described below, can be easily repeated, and involves no troublesome manipulation. The smaller part of a glass Petri dish was divided into two compartments by cementing a narrow strip of cork across the middle. A culture of luminous bacteria in nutrient gelatine was poured into one compartment, and part of the same culture containing some sheep's liver catalase into the other. When the gelatine was set, a film of chlorophyll was painted evenly over both halves, and the lid was put on. The cell was then sealed by pouring melted paraffin wax into the annular space between the rims of the dish and its cover, and gold size was then poured round on top of the wax, by which means the cell was made quite air-tight. It was placed now in a dark room, and both halves were seen to glow with equal brightness, which gradually diminished as the oxygen in the imprisoned air was used up. After two days, no glow could be detected in either half, even after 15 minutes' examination in the dark room. At this stage the cell was taken out and exposed to light for five minutes, and then brought back to the observer in the dark room, when both halves were seen to be feebly glowing, but with unequal brightness. When this glow had again ceased, the experiment was repeated with a different observer, and with the same result. On comparing notes, it was found that each observer had noticed a somewhat brighter glow in the half which contained no catalase. This result was unexpected, but it was subsequently found that the bacteria could be made to glow much more brightly by adding a drop of very dilute hydrogen peroxide than by simply exposing them to atmospheric oxygen, that is to say, they are themselves able to utilise hydrogen peroxide for the light-producing process, and in doing so derive more energy from it than from a direct supply of gaseous oxygen. This experiment therefore not only shows the production of oxygen under the conditions named, but further supports the view that this oxygen is derived from hydrogen peroxide.

(b) *In the Plant.*—The distinction between the behaviour of a plant which had been chloroformed, *i.e.* in which the enzymes had not been destroyed, and that of one in which both protoplasm and enzymes had been killed by immersion in boiling water, was emphasised in Part I, and since more than one writer has failed to confirm the observation therein recorded, that a small but significant amount of oxygen can still be evolved from a plant which has been chloroformed and subsequently exposed to light in presence of carbon dioxide, the experiment has been repeated in a different form and under more rigorous conditions. As it was essential that no trace of air should remain in the experimental vessel, the latter was exhausted very thoroughly with a small Töpler pump. Fig. 1 shows an arrangement which was found convenient in all such experiments.

The plant was contained in a wide glass tube A, which was then drawn out at the open end and sealed to a piece of narrow tubing thickened to a capillary at *a*. This tube was sealed to a T-piece leading to the pump, and carrying a tube B containing precipitated magnesium carbonate, which was employed as a source of carbon dioxide. No stopcocks or rubber connections were used. In the experiment now being considered, some *Elodea canadensis* was chloroformed for two hours, and then placed in A with water and a little thymol (used as an antiseptic). The pump was worked for some time after the last visible traces of gas had been removed, and it is certain that no air remained in the tissues. The magnesium carbonate was now heated, and the system was washed out twice with carbon dioxide. Finally A was filled with carbon dioxide at about 2 cm. pressure, and was sealed off at *a* with a small blowpipe flame. The Elodea was exposed to light for 12 hours, and the tube was then attached to the pump again and thoroughly exhausted, the gas being collected in a tube over mercury. A

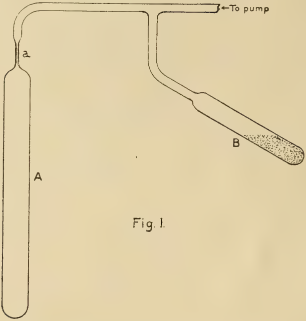

Fig. I.

small quantity (about 0·2 c.c.) of oxygen was found to be present, and was detected by absorption in alkaline pyrogallol, a result which appears to confirm the statement that the initial stage of photosynthesis can be carried on to a small extent quite independently of living protoplasm. This evolution of oxygen by dead tissues was indeed observed by Molisch* in 1904, in the case of foliage leaves of *Lamium album*, by the luminous bacteria method.

### 3. *The Absorption of Heat in a Chlorophyll Film, due to Photolysis of Carbon Dioxide.*

It has now been shown that carbon dioxide in the presence of water can be decomposed by ultra-violet light without chlorophyll, and that the same decomposition products can be obtained by the action of ordinary light when chlorophyll is present. Thus there is at least a strong probability that carbon dioxide, and not any constituent of the chlorophyll, is the parent of these decomposition-products. The remaining link in the argument has, however, been supplied by the application of a thermometric test, which is described below. It is clear that, if the assumption is correct, a chlorophyll film in an atmosphere containing moist carbon dioxide should, when illuminated, remain at a lower temperature than a similar film equally illuminated in an atmosphere devoid of that substance, for the production of formaldehyde and hydrogen peroxide from aqueous carbon dioxide involves the absorption of a large amount of heat.

The arrangement devised was a differential one, and served to show the difference between the temperatures of two chlorophyll films set up side by side in two glass tubes which contained the desired gaseous mixture. The apparatus used in the final series of measurements is diagrammatically represented in fig. 2. The chlorophyll films were painted on pieces of thin tinfoil about 1 cm. square (*a*, *a′*), which were gummed on to strips of cork (*b*, *b′*), which fitted closely in the glass tubes. A single thermo-electric junction was hammered to the back of each piece of tinfoil before the latter was fixed to the cork, and the leads from the junctions, double silk covered and soaked in shellac varnish, passed out at the top of the tubes through a narrow thickened portion of the glass, the passage being sealed by pouring melted paraffin wax into the cups at *c*, *c′*. The thermocouples were of copper constantan wire (S.W.G. No. 36), and the weight of the metal substratum of the film, including the hammered-on thermocouple, was about 0·02 grm. per square centimetre. The cork strips served to support the flimsy metal part, and to diminish to some extent the loss of heat from one surface by radiation and convection. The glass tubes into which the strips fitted were about 15 cm. long and 1·5 cm. in diameter. When an experiment was in progress the tubes were closed at their lower ends by rubber stoppers, on which rested two short tubes (*d*, *d′*), one containing a solution of carbon dioxide in water ("soda-water"), and the other a solution of potassium hydroxide; in this way the films were immersed either in an atmosphere containing moist carbon dioxide or in one devoid of it. The thermo-couples were connected differentially through a reversing key K and a suspended-coil galvanometer G. Any error due to lack of symmetry in the thermo-electric behaviour of the system was eliminated by taking always the mean of readings obtained before and after the key was reversed, and differences of temperature due to slight inequalities in the films themselves were allowed for by interchanging the solutions in the tubes *d*, *d′*. The thermocouples were calibrated directly against two mercury thermometers, so as to obtain the number of galvanometer scale divisions per degree difference of temperature.

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* 'Bot. Zeit.,' 1904, vol. 62, p. 1.

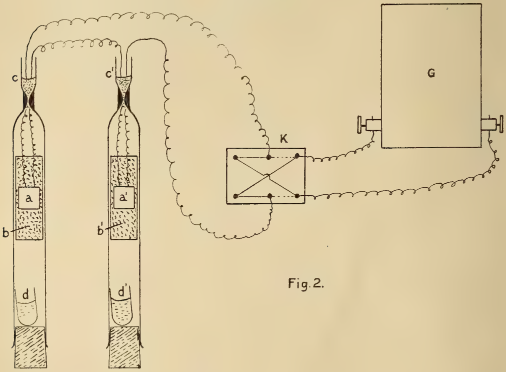

Fig. 2.

Some preliminary experiments were carried out in September, 1908, with a slightly different form of apparatus, and one series of observations is given below; the figures do not definitely settle the question at issue, as no reversal of the films was made, and an important control experiment was omitted; nevertheless they bring out several points of interest not shown in the final series.

One of the films (A) was in $CO_2$-free air, the other (B) was in a tube connected with a supply of carbon dioxide. With air in both tubes, B was 0°·56 hotter than A when both were exposed to light; in the following table of readings the observed temperature difference is corrected for this want of symmetry:—

| Time. | $t_A - t_B$. | Time. | $t_A - t_B$. |
|---|---|---|---|
| $CO_2$ slowly entering B. | | $CO_2$ in B. | |
| P.M. | ° | P.M. | ° |
| 1.30 | 0·57 | 3.22 | 0·43 |
| 1.36 | 1·42 | 3.23 | 1·00 |
| 1.40 | 1·70 | 3.24 | 1·28 |
| 1.45 | 1·56 | 3.25 | 1·42 |
| 1.48 | 0·85 | 3.26 | 1·56 |
| (Both tubes were shaded till 2.3) | | 3.27 | 1·56 |
| 2.3 | 0·14 | 3.28 | 1·42 |
| 2.4 | 0·28 | 3.30 | 1·28 |
| 2.6 | 0·43 | (Both shaded for 4 minutes) | |
| 2.10 | 0·57 | 3.34 | 0·71 |
| 2.14 | 1·00 | 3.36 | 1·28 |
| 2.20 | 1·28 | 3.37 | 1·85 |
| 2.22 | 1·28 | 3.38 | 1·56 |
| 2.24 | 0·43 | 3.39 | 1·28 |
| 2.30 | 0·14 | 3.42 | 1·14 |
| 2.32 | 0·00 | 3.44 | 1·14 |
| 2.34 | −0·28 | 3.46 | 1·13 |
| 2.36 | −0·57 | 3.48 | 1·00 |
| (At this point it was noticed that film A was almost destroyed. Two new films were therefore prepared.) | | 3.50 | 0·85 |
| | | 3.53 | 0·28 |

These figures show remarkable variations of the temperature difference with time. It will be noticed that several minutes are required for the maximum temperature difference to be established, and that this difference does not persist for more than two minutes, but gradually falls off until, if the exposure to light is continued uninterruptedly, the temperatures of the two films become equal, and ultimately the one in carbon dioxide becomes hotter than the control film in air. It was always noticed that the film in $CO_2$-free air was "scorched" and destroyed sooner than the other, and, regarding each film merely as an absorber of heat, it is obvious that the one in which the chlorophyll is more rapidly destroyed must also be the one in which the amount of heat absorbed in unit time falls off more rapidly. This probably explains the ultimate reversal of the temperature difference, for both films were being gradually destroyed, but by the time the film in carbon dioxide had lost its photolytic efficiency the one in air had become even more inefficient as an absorber of heat than the first. Again, if the films are shaded for an interval at a time when the temperature difference is diminishing, this difference begins to increase again when the exposure to light is renewed, probably because the carbon dioxide and water undergoing photolysis are used up faster than they can diffuse into the films.

A final series of measurements, in which every precaution was taken to avoid known sources of error, was made in April, 1909, with the apparatus already described. The two films were illuminated through a large ground-glass window and the vessels containing them were carefully protected from draughts. The galvanometer was a dead-beat instrument, and a movement of the spot of light over 25·4 scale-divisions corresponded to a temperature difference of 1°. The error in the readings may be taken as ±0°·01. The results are as follows—

Film A—In tube containing potassium hydroxide solution
Film B— „ carbon dioxide „
Exposed to light at 12.15 P.M.

| Time. | $t_A - t_B$. | Time. | $t_A - t_B$. |
|---|---|---|---|
| | ° | | ° |
| 12.16 | 1·30 | 2.4 | −0·31 |
| 12.17 | 1·74 | 2.5 | −0·47 |
| 12.18 | 1·78 | 2.6 | −0·51 |
| 12.19 | 1·86 | 2.7 | −0·71 |
| 12.20 | 1·81 | 2.8 | −0·79 |
| (At 12.30 the solutions were interchanged, and the films were exposed to light again at 2 P.M.) | | (Here the carbon dioxide solution was renewed) | |
| | | 2.14 | −0·67 |
| 2.1 | 0·20 | 2.16 | −0·91 |
| 2.2 | 0·00 | 2.18 | −1·03 |
| 2.3 | −0·20 | | |

From the figures given above it is evident that the temperature difference observed depends on the composition of the atmosphere surrounding the films, and that, apart from any want of symmetry in the films themselves, the one in air containing moist carbon dioxide keeps at a lower temperature than the one in air free from that gas. The transitory difference in the wrong direction observed when the films were first exposed to light after interchanging the solutions is doubtless due to a little residual carbon dioxide in film B. A possible source of error, due to a difference in the thermal properties of the gases in the two tubes, was examined by washing the chlorophyll films off their metal supports with benzene, and taking readings when the bare metal was exposed to light, the solutions being interchanged as in the film experiments. The maximum difference of temperature recorded under these conditions was 0°·06, an amount too small to affect the conclusions.

### *Conclusion.*

The present paper is concerned only with the initial stages of the assimilation process, and therefore no reference has been made to the synthesis of sugars or of starch. The particulars in which the conclusions given in Part I require modification have already been noticed—the exclusive localisation of catalase in the chloroplasts is abandoned, and also the dependence of the *post-mortem* bleaching of chlorophyll on the presence of carbon dioxide.

Finally, there now appears to be ample justification for considering that the primary products of the photolysis of aqueous carbon dioxide are formaldehyde and hydrogen peroxide; that the evolution of oxygen is due to decomposition of the latter substance by catalase; and that up to this point the process is entirely non-vital, and can be reconstructed *in vitro.*

In a paper published in 1907, A. J. Ewart ('Roy. Soc. Proc.,' B, vol. 80, p. 30) has criticised most of the experiments and all the conclusions recorded in Parts I and II. As it is impossible to answer all the objections within the limits of a short paper, replies to a few of the more important are briefly indicated below. (i) The experiments upon which Ewart bases his opinion that formaldehyde was not produced as a decomposition-product of carbon dioxide under the conditions named in Parts I and II are quite valueless for that purpose, because he used the reagent (decolourised rosaniline) in such a way that a colouration would inevitably be produced in the material to be tested. Since the sulphur dioxide (used to keep the rosaniline decolourised) escapes from the solution on warming, or when a large surface (compared with the volume) is left exposed to the air, the method employed and described by Ewart (pp. 30—31) is clearly inadmissible. It may be as well to state here that all the materials—gelatine, petroleum, ether, etc.—used for the experiments described in Part II were tested with the same specimen of Schiff's reagent which was afterwards used to detect formaldehyde, and were only employed if found to be initially free from that substance. In view of the more recent experimental work of Schryver referred to in this paper, it seems unnecessary to discuss the subject at greater length here. (ii) The phenomenon of the bleaching of chlorophyll, and its explanation, have already been dealt with (p. 104). (iii) With regard to the production of hydrogen peroxide, Ewart is mistaken in supposing that we were "unaware that the absence of hydrogen peroxide from living cells has been definitely established": on the contrary, it was expressly stated that an enzyme was present whose function was to decompose that substance as fast as it might be formed. Since chlorophyll is itself attacked (bleached) by hydrogen peroxide, the latter has also escaped detection when the enzyme has been destroyed. (iv) The extra-cellular evolution of oxygen.—The experiment described in Part II has been misrepresented in important particulars by Ewart (p. 34), and in his attempt to repeat it the conditions of the original experiment were not observed. (v) The simultaneous production of formaldehyde and hydrogen peroxide is objected to (p. 35) on the ground that these two substances under certain conditions interact, and form carbon dioxide and hydrogen (or, ultimately, carbon dioxide and water). The difficulty, however, is imaginary, and the result is possible, because (*a*) the position of equilibrium in the reversible change $CO_2 + 3H_2O \rightleftarrows HCHO + 2H_2O_2$ is displaced towards the right by the addition of light energy, and (*b*) the process is continuous so long as the products on the right-hand side are removed, as in a living plant they are.

H. Euler ('Zeits. für physiol. Chemie,' 1909, vol. 59, p. 122) supports Ewart's criticisms, without, however, giving any particulars (*cf.* foregoing paragraph). He also mentions some experiments with solutions of chlorophyll, quinine sulphate, and fluorescein, which gave negative results. This agrees with our own experience, so far, at least, as chlorophyll solutions are concerned.

Mameli and Pollacci ('Atti dell' Ist. Bot. dell' Univ. di Pavia,' Series II, vol. 13) have published a critical memoir in which, in the first place, they re-affirm the possibility of detecting formaldehyde in the living plant: this appears now to be fully confirmed by Schryver (*loc. cit.*). These authors also failed to observe any evolution of oxygen *in vitro* when they repeated the experiment already referred to, but it is possible that, as they stated that they were unable to prepare a specimen of chlorophyll free from formaldehyde, this substance may have interfered with the action of the catalase in contact with the film of chlorophyll, in which case no oxygen would be produced.

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# *Transmission of Amakebe by means of* Rhipicephalus appendiculatus, *the Brown Tick.*

By Dr. A. Theiler, C.M.G., Pretoria.

(Communicated by Colonel Sir D. Bruce, C.B., F.R.S. Received April 20,—Read May 18, 1911.)

That the disease in calves of Uganda called Amakebe is identical with East Coast fever had to be concluded after the presence of the so-called blue bodies of Koch, or plasma bodies, had been demonstrated in the internal organs; these bodies represent certain stages, agametes, agamonts, and gamonts, in the life cycle of *Theileria parva*. Accordingly, it had to be expected that Amakebe could be transmitted by means of such ticks, which act as hosts for this parasite. The most common tick of Uganda is the Brown Tick *Rhipicephalus appendiculatus*, which has been proved in South Africa to be the principal transmitter of East Coast fever.

When in Uganda in 1909 an arrangement was made between Mr. Hutchins, the Government Veterinary Surgeon of Uganda, and myself, to place adult brown ticks, collected as nymphæ from calves suffering from Amakebe, on susceptible calves in my laboratory in Onderstepoort, Pretoria, Transvaal; these ticks were to be collected by Mr. Hutchins as opportunity occurred.

On several occasions Mr. Hutchins forwarded me brown ticks, which he had placed in a glass tube; in every instance they arrived alive and in good condition, having moulted in transit from the nymphal into the adult stage. The first two lots of ticks failed to transmit the disease, the nymphæ probably having been collected off calves which had recovered from the disease, when the blood no longer contained the pathogenic parasite. Experiments with the last lot were successful, as will be shown hereunder.

### EXPERIMENT TO NOTE WHETHER BROWN TICKS COLLECTED AS NYMPHÆ IN UGANDA FROM A CALF SUFFERING FROM AMAKEBE WILL TRANSMIT THE DISEASE TO SUSCEPTIBLE CALVES IN THE TRANSVAAL.

(1) *Bull calf* 1118, born and reared in Onderstepoort, was infested on January 23, 1911, with 10 adult brown ticks, forwarded by Mr. Hutchins from Uganda and received here on January 4, 1911. All 10 ticks were found attached to the calf the following day.

The calf showed almost immediately a rise of temperature, developing into a definite curve, during which the so-called marginal points (*Anaplasma marginale*) were noted to be present in great numbers; this curve was typical of the disease anaplasmosis, and the blood lesions found were those of an oligocythæmia (anisocytosis, poikilocytosis, polychromasia and basophilia) which followed as a sequel. The temperature gradually dropped, and the calf was found dead on the 22nd day after tick infestation. An examination of the lymphatic glands was made on the 17th day, and a negative result was registered.

*Post-mortem Examination on Calf* 1118.—The condition was fair. Rigor mortis was present. Tympanitis was noted. The lungs were partially collapsed and showed some atelectatic areas. On section a slight œdema became noticeable; in the trachea was some foam. The bronchial lymphatic glands were swollen, the mediastinal glands were normal.

The pericardium contained some clear liquid. The blood in the ventricles was well coagulated. Both endocards were normal. The liver was enlarged and had a mottled appearance due to small pale areas; the parenchyma was soft. The periportal lymphatic glands were enlarged. The bile was yellow and viscid. The spleen was enlarged, measured 30 cm. by 10 cm., the pulp was softened, jam-like; the trabeculæ were indistinct. All four stomachs were normal.

The mucosa of the jejunum was slightly thickened and œdematous, that of the cæcum and colon was slate-coloured and contained a small number of disseminated parasitic nodules.

The kidneys were pale, the capsule was easily detachable and the urine was clear. The exterior lymphatic glands were swollen.

The microscopical examination of the blood proved the absence of any parasites. In the lymphatic glands the so-called plasma bodies of Koch were found and described as rather small, viz., agametes and young agamonts (according to Gonder*); the same observation was made in preparations of the spleen.

*Diagnosis.*—East Coast fever.

(2) *Calf* 1143.—On February 14, 1911, this calf was infested with 10 adult brown ticks of the same lot obtained from Uganda. On February 15 seven of these ticks were found attached. After an incubation time of 13 days a typical fever curve ensued, which, however, never reached high records. The animal died on the 24th day.

On the 15th day after the tick infestation both blood and glands were examined and the result was negative. The examination on the 17th day revealed rare agamonts in the prescapular glands, but no parasites in the blood; on the 20th day both agamonts and gamonts were found in the lymphatic glands in a fair number, and *Theileria parva* was frequently met with in the red corpuscles.

*Post-mortem Examination of Calf* 1143.—Rigor mortis was present. The condition was rather poor. All external lymphatic glands were very much swollen. The lungs had not collapsed; there were some patches of red hepatisation in right anterior lobe and a small area in the left lobe. The lesions of hyperæmia and œdema were pronounced. There was a fibrinous coagulum in the trachea.

The bronchial and mediastinal lymphatic glands were enlarged and œdematous. The heart contained coagulated blood. Both the endocardium and the myocardium were normal. The liver was enlarged, the margins were rounded, the colour was reddish brown, the parenchyma was rather soft. The bile was green, thick, and viscid.

The spleen measured 30 cm. by 9 cm.; the pulp was soft and jam-like, and the trabeculæ were indistinct. The mucosa of the fourth stomach was slate-coloured; there were a few small hæmorrhagic ulcers. The mucosa of the jejunum showed longitudinal slate-coloured streaks. The mucosa of the ileum was slightly thickened, and dotted with punctiform hæmorrhages. The mucosa of the cæcum was thickened, the blood-vessels were injected, and there were patches of hyperæmia. The mucosa of the colon was slightly swollen and slate-coloured. The mesenteric glands were much enlarged and rather soft. The kidneys were rather pale; the boundary zone of the right kidney was slightly hyperæmic; the capsule was easily detachable. The bladder contained clear, yellow urine.

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* 'Annual Report of the Government Veterinary Bacteriologist, Transvaal, S.A.,' 1909—10.

*Microscopical Examination.*—Koch's granules were found frequently in the lymphatic glands and spleen.

*Diagnosis.*—East Coast fever.

The infestation of two calves with adult brown ticks collected as nymphæ in Uganda from a calf suffering from acute Amakebe, was succeeded in both instances by a fatal disease, which could be diagnosed as East Coast fever from the appearance of the so-called Koch's blue bodies or plasma granules, which represent, according to Gonder, the agametes, agamonts, and gamonts in the life cycle of *Theileria parva.* The *post-mortem* examination corresponds with Amakebe of Uganda, and with what is known as East Coast fever. The fact that the blood of the first calf did not show blood parasites is nothing unusual in Amakebe. The agamonts were there, no gamonts had yet developed, accordingly no gametes of *Theileria parva* could be found. This calf apparently died at the beginning of the disease, the animal being weakened by the preceding *anaplasma* inoculation. The second calf represented in every respect a typical case of East Coast fever.

## CONCLUSION.

Amakebe of Uganda is identical with East Coast fever of South Africa, and is transmitted by the tick *Rhipicephalus appendiculatus.* This conclusion corroborates that obtained by the Royal Society Sleeping Sickness Commission of 1909.

# *The Discrimination of Colour.*

By F. W. Edridge-Green, M.D., F.R.C.S., Beit Medical Research Fellow.

(Communicated by Prof. W. M. Bayliss, F.R.S. Received April 24,—Read May 18, 1911.)

(From the Institute of Physiology, University College.)

In a paper on the relation of light perception to colour perception,* and in previous writings,† I have stated that if a portion of the spectrum be isolated, it will appear monochromatic, the length of the monochromatic region varying with the intensity and wave-length of the light and the colour perception of the observer. Most normal sighted persons make about eighteen such divisions in a bright spectrum.

In a paper in the 'Proceedings of the Royal Society,'‡ Lord Rayleigh, whilst agreeing that the facts were as I stated in the conditions described by me, expressed the opinion that he could distinguish between the wave-lengths included in a monochromatic division to the extent of discriminating between the colours of the two D lines. Lord Rayleigh kindly lent me the colour box with which he had made the experiments, and, on repeating them in the manner described by him, I arrived at similar results. I hope, however, to be able to show that the results obtained by Lord Rayleigh were due to the admixture of small quantities of white and coloured light and to certain physiological influences which had not been taken into consideration, and which prevented him from arriving at a correct interpretation of the colours.

If a prism, even of the finest polish, be examined with a strong light against a dark background, numerous small particles and irregularities of the surface, which irregularly disperse the light, will be seen. The reflections from the sides of the prisms, lenses, and sides of the box have also to be taken into consideration. The amount of this irregularly dispersed light is small, but is a very important factor taken in conjunction with other facts. It is necessary, therefore, in order to get rid of the greater part of this irregularly dispersed light, to allow the light included in a monochromatic region to pass through a second aperture, such as that in my spectrometer. When this is done, I have found it impossible by any method which I have adopted to distinguish between the various waves included in the monochromatic region. I have magnified the image of this region with eyepieces of different power, making a corresponding increase in the light to make up for the loss of luminosity caused by the magnification. I have also obstructed the central portion of the monochromatic region with a screen, and the remaining portions have still appeared monochromatic. The most conclusive experiment, however, is the examination of the monochromatic region with an achromatic double image prism, the intensity of the source of light being increased as before. By this method two rectangular monochromatic fields are seen, and can be arranged so that they are side by side and just touch. The portion belonging to the red side of the spectrum of one can be made to touch the portion belonging to the violet side of the spectrum of the other. This position is therefore most favourable for the detection of any difference, and yet I cannot detect any, neither can any other observer to whom I have shown the experiment. The experiment can be observed objectively in the following manner: An arc light being used for the illuminating source, a pure spectrum is obtained; a portion of this spectrum, forming a monochromatic region, is allowed to pass through an adjustable slit. Two images of this monochromatic region can be thrown with the aid of a double image prism upon a screen and made of any required size. The varying size of the monochromatic regions with different persons can by this means be demonstrated to a large number.

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* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 458.

† 'Colour Blindness and Colour Perception,' International Scientific Series.

‡ December, 1910.

Another point which I found with Lord Rayleigh's apparatus is the difficulty of obtaining both fields of similar intensity. The slightest movement of the eye also causes an alteration in the number and kind of rays which enter the eye. When two fields are of unequal intensity the physiological effect of contrast is evoked, which causes an erroneous judgment of the colours under observation. In fact, weak orange light may, by contrast with bright red light, appear green. In making experiments on the discrimination of colour, the rays of light from the two regions to be compared should strike the eye at as nearly as possible the same angle; the fixation point of the eye should be in the centre between the two regions, so that one region may not be more influenced by the pigment of the yellow spot, or the blood in the retina, than the other; and equal amounts of light from each region should enter the eye.

The importance of the irregularly dispersed light in association with contrast in dealing with questions of colour has been overlooked by many physicists, as several instruments have been constructed for the investigation of colour and colour-vision, which are defective on this ground. It was this irregularly dispersed light, as shown by Helmholtz,* which caused the apparent change in the colours of the spectrum observed by Brewster, and which led him to suppose erroneously that there were three kinds of solar light.

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* 'Poggendorff's Annalen,' 1852, No. 8.

In conclusion, when special means are taken to have as pure a spectrum as possible, I can find no method which will enable me to distinguish as distinct colours the wave-lengths in a monochromatic region. I therefore regard the appearance of the monochromatic region as a fundamental physiological fact, as I stated over 20 years ago.

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# *Note on the Sensibility of the Eye to Variations of Wave-length.*

By W. Watson, D.Sc., F.R.S.

(Received June 12,—Read June 29, 1911.)

In a recent communication to the Royal Society, Dr. Edridge-Green has suggested that the reason Lord Rayleigh found he was able to distinguish a difference in hue between two monochromatic patches of yellow (D) light, when they differ in wave-length by about the distance between the sodium lines (0·6 $\mu\mu$), is that (*a*) the spectrum used was not pure, and hence the patches were not monochromatic; and (*b*) that the difference in wave-length was apparent because of admixture with white light. Some experiments made by the author seem so conclusively to show that at any rate the second of the above reasons cannot be correct that it seems worth while to put them on record.

By means of Sir William Abney's double spectrum apparatus,* two patches of monochromatic light were thrown side by side on a magnesium carbonate screen, and matters were so arranged that no line of separation was observable when the patches were of the same colour. Each patch was 9 mm. by 18 mm., and the observer was at a distance of 60 cm. The intensity of the illumination on the screen was throughout 3·5 candle-metres. The slit in the second spectrum apparatus was kept at a fixed point in the spectrum, while that in the first spectrum was moved by means of a micrometer screw, the movement being read on a scale on which a millimetre represents in the yellow a difference in wave-length of 3·7 $\mu\mu$.

By cutting off the light from one slit and placing a short focus lens in front of the other an enlarged image of the slit would be formed on the screen. Thus by watching this image and gradually opening the slit the width of the Edridge-Green monochromatic patch could be determined.

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* 'Phil. Trans.,' A, 1905, vol. 205, p. 333.

Then, the lens being removed and the slits in the two spectra adjusted so that their width corresponded to 3 $\mu\mu$, the movement of the first slit necessary to produce an observable difference in hue, first in one direction and then in the other, was determined. In this way the two sets of observations were made under exactly the same conditions as to illumination, size of image on the retina, state of dark adaptation, etc. When determining the change in wave-length required to produce an observable difference in hue, the slit was always moved till the difference in hue was *quite distinct*, and no hesitation was felt as to which patch was, say, on the red side. In general, the observer did not move the slit, so that he did not know in which direction the change in colour would take place.

Fourteen observers were tested in the above manner, and their readings, which agree very well together, give the following mean values :—

Sodium Light.

| | |
|---|---|
| Width of monochromatic patch .................................... | 4·5 $\mu\mu$. |
| Difference in wave-length easily detected as a change in hue | 1·4 $\mu\mu$. |

It will be observed that there is a very marked difference, and that when the eye is not dealing with a continuous variation in hue, as is the case when a portion of the spectrum is observed, a very much smaller difference in wave-length is apparent as a difference in hue, and this even when the conditions are as nearly as possible identical.

This point was also investigated in a somewhat different manner. The monochromatic patch having been projected on the screen, a rod was interposed so as to cut out a portion corresponding to 1·9 $\mu\mu$ difference in wave-length from the middle, and the two remaining portions were then brought together by means of a Fresnell biprism. When this was done the difference in hue was most marked, though on removing the rod the patch again looked monochromatic.

An attempt to repeat Dr. Edridge-Green's experiment, using a biprism, failed; since there was sufficient polarisation of the light produced by the prisms of the spectroscope to cause such a difference in brightness of the two images as to mask any difference in hue.

To investigate what effect, if any, the presence of white light mixed with the colours would produce on the perception of a difference of hue, arrangements were made by which a known amount of white light fell on both halves of the screen. Different quantities of white were added, and in the following table 100 parts of white were of the same luminosity as the colour in each case. Observations were made with yellow (D), red and green light. No difficulty was found in making the measurements in the yellow, where the luminosity of the spectrum is nearly a maximum, and hence a slight movement of the slit causes no appreciable change in the brightness. In the red and green, however, a movement of the slit causes a much more apparent change in brightness than of hue. The method adopted to make the settings was to move the slit till no change in brightness obtained by opening or closing the slit would cause the two patches of colour to look the same.

Table.

| Wave-length of colour used. | Amount of white added (100 = luminosity of coloured light). | Change in wave-length which produced a clearly observable difference in hue. |
|---|---|---|
| μμ. | | μμ. |
| 589 | 0 | 1·55 |
| | 10 | 1·55 |
| | 20 | 1·6 |
| | 40 | 1·8 |
| | 80 | 2·05 |
| | 160 | 2·5 |
| | 320 | 3·95 |
| 632 | 0 | 5·8 |
| | 10 | 5·8 |
| | 20 | 6·3 |
| | 40 | 7·7 |
| | 80 | 9·6 |
| | 160 | 15·0 |
| 527 | 0 | 6·1 |
| | 10 | 6·0 |
| | 20 | 6·3 |
| | 40 | 7·5 |
| | 80 | 8·9 |
| | 160 | 13·7 |

It will be observed that in the case of yellow (D) light, there is no observable effect on the minimum change in wave-length required to produce an observable change in hue produced by small additions of white light. Even when the luminosity of the added white is three times the luminosity of the coloured, the minimum change in wave-length observable is decidedly less than the extent of the monochromatic patch.

In the case of the red also, the addition of white does not increase the sensitiveness of the eye to changes in wave-length. With the green, however, a slight increase of sensitiveness appears to be produced by the addition of a small quantity of white. The effect, however, is very small, and, owing to difficulties due to change in luminosity referred to above, is hardly measurable. By allowing the white to only fall in half each of the patches, and noticing whether a small movement of the slit caused a greater difference in hue, with or without white, the above results were confirmed, *i.e.*, in the case of yellow and red, no increase due to the white was observable, and in the case of the green a small addition of white seemed to make the difference in hue very slightly more pronounced.

The above observations seem to indicate that difference in our power of appreciating differences in hue, according as we are comparing two monochromatic patches, or a single patch in which the hue changes gradually from one side to the other, is not due to admixture of white light.

# *On the Direct Guaiacum Reaction given by Plant Extracts.*

By Miss M. Wheldale, Fellow of Newnham College, Cambridge.

(Communicated by W. Bateson, F.R.S. Received April 25,—Read May 18, 1911.)

In the literature dealing with oxidising enzymes considerable attention has been drawn to the fact that the juices of some plants blue guaiacum tincture directly (direct action), whereas the juices of other plants only bring about the blueing on addition of hydrogen peroxide (indirect action).

As an explanation of this phenomenon Chodat and Bach formulated a hypothesis which has, in part, been generally accepted. These authors maintain that direct blueing of guaiacum is brought about through the activity of a system consisting of oxygenase, peroxide, and peroxidase. The peroxidase is an enzyme capable only of transferring oxygen from the peroxide to the guaiacum. The peroxide, after reduction, is again re-oxidised by a second enzyme, the oxygenase. The juices of such plants as give a direct action contain, according to Chodat and Bach, all three components of the system. In others, the peroxidase alone is present, and hence the guaiacum cannot be oxidised until a peroxide, such as hydrogen peroxide, is artificially supplied.

In a recent paper Moore and Whitley* have cast considerable doubt upon the existence of any such enzyme as an oxygenase and give experimental evidence as proof of the view that all plants contain a peroxidase, but only those give a direct action of which the tissues contain more or less organic peroxide.

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* Moore and Whitley, "The Properties and Classification of the Oxidising Enzymes," 'Biochem. Journ.,' vol. 4, 1909.

The experiments I have made with oxidising enzymes corroborate these doubts. I have found that the power to give the direct guaiacum action in any plant is always accompanied by another phenomenon, *i.e.* the formation of brown or reddish-brown pigment when the tissues are injured mechanically or are subjected to chloroform vapour.

Both phenomena are peculiar to certain genera, other genera giving the indirect action only and being unaffected in the same way by injury or by exposure to chloroform vapour. On the whole the direct action is especially characteristic of the Compositæ, Umbelliferæ, Labiatæ and Boraginaceæ, and certain genera of the Scrophulariaceæ, Rosaceæ, Leguminosæ, Ranunculaceæ, and of many other natural orders. It is absent from or rare in the Cruciferæ, Caryophyllaceæ, Crassulaceæ, and Ericaceæ.* The direct action is also more frequent among the Dicotyledons than the Monocotyledons.

The results of my observations have led to the conclusion that the direct action given by the extracts of the plants I have examined† is due to the presence of the dihydric phenol, pyrocatechin, in the tissues of the plants.

That the darkening of plant juices is due to the presence of pyrocatechin has been previously suggested by Grafe.‡ The same suggestion has also been made by Weevers§ in connection with his work on the relationship between pyrocatechin and salicin in *Salix* and *Populus.*

Pyrocatechin rapidly oxidises on exposure to air, and then acts as an organic peroxide, enabling the peroxidase, which is almost universally present, to transfer oxygen to the guaiacum. The plants, such as I have examined, from which pyrocatechin is absent do not give the direct action.

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* A similar distribution of direct action has been noted by Passerini, "Sulla presenza di fermenti zimici ossidanti nelle piante fanerogame," 'Nuov. Giorn. Botan. Ital.,' 1899. Also by Clark, "The Plant Oxidases," 'Dissertation,' New York, 1910.

† The plants examined included *Aconitum Napellus*, *Caltha palustris*, *Helleborus fœtidus*, *Mahonia aquifolium*, *Chelidonium majus*, *Prunus Laurocerasus*, *Pyrus japonica*, *Cornus mas*, *Chærophyllum sylvestre*, *Ligustrum vulgare*, *Anchusa officinalis*, *Myosotis dissitiflora*, *Rosmarinus officinalis*, *Viburnum Opulus*, *V. Tinus*, *Sambucus nigra*, and *Taraxacum officinale*, in all of which pyrocatechin is present. Pyrocatechin was not detected in *Crocus vernus*, *Galanthus nivalis*, *Arabis albida*, *Eranthis hyemalis*, *Cheiranthus Cheiri*, *Brassica oleracea*, *Viola odorata*, *Primula acaulis*, *Iris germanica*, *Lupinus sp.*, *Narcissus Pseudo-narcissus*, nor in *Arum maculatum*.

‡ Grafe, "Über die Dunkelfärbung von Rübensäften,' 'Oest. Zeitsch. f. Zuckerindus. u. Landwirtschaft,' 1908.

§ Weevers, "Die physiologische Bedeutung einiger Glykoside." Extrait du 'Recueil des Travaux Botaniques Néerlandais,' vol. 7, 1910.

These conclusions are based upon three observations:—

(1) Pyrocatechin can be detected by the green reaction with ferric chloride (subsequently purple and red on addition of dilute sodium carbonate) in extracts from plants giving both the direct action and a brown pigment on exposure to chloroform vapour. The alcoholic extract of the plants is evaporated to dryness, and the pyrocatechin extracted with ether or acetone after the removal of chlorophyll and other substances soluble in chloroform. Pyrocatechin was not detected in any appreciable quantity in plants giving the indirect action only.

(2) After evaporation, the ether extract containing pyrocatechin will bring about in many cases a direct blueing of guaiacum when added to a solution containing peroxidase only.* Care must be taken to neutralise the residue (if acid) after evaporation of the ether and before addition of the peroxidase and guaiacum.

(3) When a slightly alkaline solution of commercial pyrocatechin is allowed to stand in air, oxidation takes place and a brown colour is developed. Such a solution added to a peroxidase solution and guaiacum tincture brings about a blueing of the guaiacum. Similar experiments were made with phenol, resorcinol, hydroquinone, pyrogallol, and phloroglucin; also with benzoic, salicylic, *m*-oxybenzoic, *p*-oxybenzoic, protocatechuic, gallic, and tannic acids, and quercetin.

A positive result was obtained with protocatechuic acid only. There is therefore probably a connection between the ortho-position of the hydroxyl groups and the specific capacity of these substances as regards their power to activate the peroxidase. According to Czapek,† protocatechuic acid rarely occurs free in the plant, but further experimental investigation would be necessary to establish this point.

Hence we may conclude that the direct action of certain plant extracts is due to the *post-mortem* oxidation of a definite metabolic product, and the action as such has probably no significance in the metabolism of the living plant.

There is some evidence in favour of the supposition that the pyrocatechin exists as a glucoside, and that the hydrolysis of this compound into sugar and phenol is accelerated by injury or chloroform vapour. In many cases very little oxidation takes place in the alcoholic residue obtained by plunging the leaves of a pyrocatechin-containing plant into boiling alcohol and thereby preventing decomposition of the glucoside. If, however, such a residue is boiled with dilute acid in order to hydrolise the glucoside, considerable oxidation, accompanied by brown coloration, will take place.

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* Extract of white Brompton Stock was used for this purpose. The guaiacum tincture was always boiled with animal charcoal before using, as recommended by Moore and Whitley, *loc. cit.*

† Czapek, 'Biochemie der Pflanzen.'

Palladin* maintains that the formation of *post-mortem* pigments from aromatic chromogens is proof of the significance of the latter in respiration. Some of the plants from which he obtained the greatest quantity of pigment by treatment of the extracts with peroxidase and hydroxyl are of the pyrocatechin-containing type, and the presence of this phenol, when it occurs, would doubtless accelerate the oxidation of the extracts. But the reactions obtained after death may be no real guide to knowledge of the true metabolic reactions of the living tissues.

The formation of brown pigment on autolysis and injury in pyrocatechin-containing plants is no doubt largely due to the oxidation of the phenol itself, but, in addition, coloration may be caused by the oxidation of other aromatic compounds, *i.e.* tannins, flavones, etc., when once the system peroxide-peroxidase has been established.

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# *The Action of Radium Radiations upon Some of the Main Constituents of Normal Blood.*

By Helen Chambers, M.D., and S. Russ, D.Sc., Beit Memorial Research Fellow.

(Communicated by Dr. J. R. Bradford, Sec. R.S. Received May 1,—Read June 1, 1911.)

The following experiments were undertaken with a view to determining the effect *in vitro* of the different radiations from radioactive substances upon some of the main constituents of normal blood. The observations have so far been extended to the hæmolytic action of the $\alpha$-rays on red corpuscles, to the effect of these rays on leucocytes, and to their action on opsonin and complement. Numerous experiments have also been made with the $\beta$- and $\gamma$-rays, but, generally speaking, the results have been of a negative character.

## *The Hæmolytic Action of the Emanation.*

When radium emanation is mixed with citrated human blood, hæmolysis results. The liberation of hæmoglobin is a gradual process, as is evidenced by the following experiment, which is typical of several:—A glass bulb of volume 30 c.c. contained 2 c.c. of citrated blood and emanation equal in quantity to the equilibrium value of 26·5 mgrm. $RaBr_2$. The blood was examined at different times and a count made, by means of a Thoma Zeiss apparatus, of the percentage of completely hæmolysed corpuscles. The results are indicated in Table I.

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* Palladin, "Über das Wesen der Pflanzenatmung," 'Biochem. Zeitsch.,' 1909.

Table I.

| Time of exposure. | Completely hæmolysed corpuscles. Percentages. |
|---|---|
| hours. | |
| 2 | 0 |
| 6 | 0 |
| 19 | 7·2 |
| 42¾ | 84 |

When hæmolysis was complete, the corpuscles were found to be colourless and slightly shrunk, but retaining their corpuscular form. The spectrum of met-hæmoglobin was observed at the end of the observations. The conversion from oxy-hæmoglobin was not complete, however, as some of the bands of this substance were also visible.*

The hæmolysis was found to be due to a direct action of the $\alpha$-particles on the red corpuscles by the following experiments:—

(1) Emanation mixed with washed red corpuscles gave marked hæmolysis in 24 hours.

(2) Emanation mixed with serum for 24 hours and the latter added to washed red corpuscles gave no hæmolysis.

(3) Emanation enclosed in a glass tube just thick enough to exclude the $\alpha$-rays, while allowing free exit of the $\beta$- and $\gamma$-radiations, produced no hæmolysis in blood contained in a tube which surrounded that in which the emanation was held.

The concentration of the emanation in these three experiments was nearly the same as in that initially described. The direct proof of the hæmolysis being due to the $\alpha$-particles has been shown by means of the apparatus of fig. 1.

A finely powdered specimen of radium bromide was spread over a circular area of 2 sq. cm., this being the bottom of a cavity 1 mm. deep in a brass capsule (fig. 1). The grains were held in position by very thin varnish. The cavity was covered with an air-tight sheet of mica (A), sufficiently thin to allow escape of the $\alpha$-particles.*

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* Hæmolysis and the formation of met-hæmoglobin have been observed by Henri and Mayer ('Comptes Rendus,' 1904, p. 521) experimenting with frog's blood and that of the dog, by exposing it to 100 mgrm. of radium. The type of radiation producing the results is, however, not stated.

On another thin sheet of mica (B) a drop of citrated blood (C) was spread over a known area. The drop was covered over with a shallow watch-glass (E), vaselined round its edge to prevent evaporation. The mica B was then placed over A, and radiation proceeded for any desired interval.

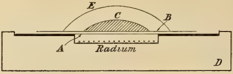

FIG. 1.

It was clear that, since the liberation of hæmoglobin is a gradual process, if an accurate relation between the time of radiation and the number of hæmolysed corpuscles were to be found, sufficient time must elapse after radiation and before the count was made, to allow of the release of the hæmoglobin from the affected corpuscles. Twenty-four hours were found to be sufficient for this purpose.

The same volume of citrated blood (12·5 c. mm.) was taken each time and spread over an area of 1·5 sq. cm. on a sheet of mica. This was then exposed to the $\alpha$-radiation from the radium capsule. A control was provided in each case.

It may be seen from Table II and the curve in fig. 2 that the number of totally hæmolysed corpuscles for a given intensity of radiation bears a simple relation to the time of exposure. A separate experiment showed that the products of hæmolysis had no hæmolytic action on other red corpuscles.

Table II.

| Time of exposure. | Percentage of unhæmolysed corpuscles. |
|---|---|
| h. m. | |
| 0 15 | 98 |
| 1 0 | 93·5 |
| 1 40 | 84·5 |
| 4 15 | 58·8 |
| 6 35 | 45·6 |
| 10 45 | 25 |
| 24 0 | 10 |

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* Two such brass capsules were made, one containing 2·4 mgrm., the other 3·27 mgrm. $RaBr_2$. We are indebted to Mr. F. H. Glew for their preparation.

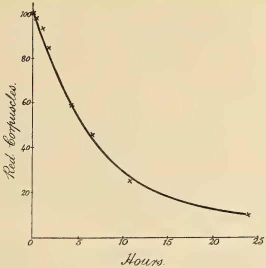

FIG. 2.

An estimate of the number of α-particles required to hæmolyse a red corpuscle is possible owing to the precision with which the essential quantities in the calculation are known, viz., the number of corpuscles per cubic millimetre of blood, and the number of α-particles emitted per second from a measured quantity of emanation.*

From experiments in which the emanation was mixed with blood, a calculation results in the number 2000 being required for the complete hæmolysis of a red corpuscle. From those in which the α-particles had to penetrate two sheets of mica, a maximum estimate of the number in question is 8000. In view of the different experimental conditions, the difference between the two numbers is not significant.

### *The Action of the α-Rays on Leucocytes.*

It has been found that the α-particles are capable of not only destroying leucocytes, but also, by virtue of their action on the serum, rendering a radiated region free of them.

A simplification of Ponder's† method of obtaining leucocytes has been used for these experiments. A drop of blood is put on a mica plate, covered, but not touched, by a watch-glass to prevent evaporation, and incubated at 37° C. for about 20 minutes. On removal of the clot large numbers of leucocytes are found on the surface of the mica, to which they adhere firmly; they may be repeatedly washed and then stained without being freed.

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* Rutherford and Geiger, 'Roy. Soc. Proc.,' A, 1908, vol. 81, p. 173.

† Ponder, 'Camb. Phil. Soc. Proc.,' 1909, vol. 15, Part I.

If the blood be not incubated, clotting is delayed, and the motion of the leucocytes to the surface considerably prolonged; this is of importance in the following experiments:—

A drop of blood was placed on a clean piece of mica, sufficiently thin to allow easy penetration of the $\alpha$-particles. It was covered with a watch-glass and placed on the radium capsule, the radiation from which was screened in such a manner that it was entirely confined to a square window of about 1·5 sq. mm. On placing the mica sheet over the capsule, therefore, the drop of blood was not radiated by the $\alpha$-rays except the area which was directly above the small window.

After an exposure of about 20 hours *at room temperature* the clot was removed, the mica surface washed with saline, and stained. The resulting picture was as indicated by fig. 3.

It is seen that in a region corresponding to the radiated area there is an almost complete absence of leucocytes; the area free of leucocytes is, however, slightly greater than that of the window, the ratio determined by a magnified projection on squared paper being 1·34 : 1. This increase is probably due to cross firing of the rays. A different result was obtained under the following circumstances:—

A drop of blood was shed on to a thin mica sheet, covered with a watch-glass, and *incubated for 1½ hours.* This ensures a plentiful supply of leucocytes on the mica surface.

The system was removed from the incubator and placed over the radium capsule, which was again provided with a small square window, of area 1 sq. mm., through which came the $\alpha$-rays. After an exposure of about 20 hours at room temperature, followed by the usual staining process, the picture presented was as shown in fig. 4.

Inspection showed that the area free of leucocytes was much larger than that of the window. A measurement similar to that already described gave the ratio 2·8 : 1. In this experiment numerous degenerate leucocytes were observed in the radiated and surrounding zones.

If therefore the $\alpha$-radiation proceeds during the slow migration of the leucocytes out of the clot to the mica at room temperature, the area free of leucocytes practically corresponds to the aperture through which the rays come. If, however, the leucocytes are first allowed to make their way to the mica surface, part of which is then radiated, the area free of leucocytes is found to be much larger than that of the radiated area.

The different results in these two experiments indicate that in the former the leucocytes do not reach a radiated region, but tend to drift into a protected zone. The leucocytes do not, however, move as a direct result of the $\alpha$-radiation, for if incubation occur simultaneously with radiation, leucocytes are found on the radiated surface, although to a modified extent.

During the slow motion of leucocytes to the mica at room temperature, changes are taking place in the radiated serum, forming a layer over the mica. As will be seen later, there is a lowering of the opsonin and complement content of the serum over the radiated region.

The leucocytes seem to move from a radiated to a non-radiated region, *i.e.*, from a serum in which changes have been induced by the radiation, to a serum which is unaltered. This motion can be explained by changes in surface tension corresponding to some alteration in the constitution of the fluids in which the leucocytes are moving.

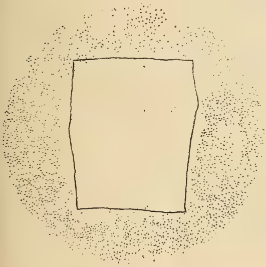

Fig. 3.

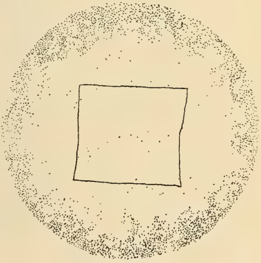

Fig. 4.

The surface tension of $\alpha$-radiated serum, determined by the capillary tube method, showed a reduction when compared with that of a control normal serum. The change is indicated by the figures in Table III.

Table III.—Surface Tension in dynes per cm. at about 10° C.

| Normal serum. | Radiated serum. | Time of radiation. |
|---|---|---|
| | | hours. |
| 66·2 | 63·2 | 24 |
| 66·2 | 61·2 | 44 |
| 67·5 | 59·1 | 54 |

Further evidence of the absence of leucocytes upon a radiated surface and of their drift into a protected region is given by fig. 5, which was obtained by screening the $\alpha$-radiation from one-half of the capsule. The motion of the leucocytes occurred at room temperature.

To account for the enlargement of the area free of leucocytes when incubation occurs previous to radiation, the suggestion is put forward that the leucocytes are destroyed after making their way to the mica surface and liberate some fluid products which have a destructive effect upon the leucocytes in the surrounding zones. These products gradually diffuse away from the radiated area and effect the destruction of leucocytes quite outside the direct stream of the $\alpha$-rays.

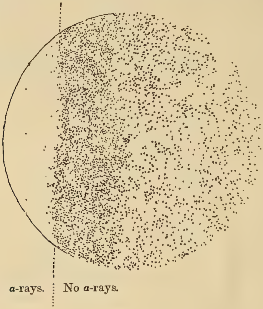

FIG. 5.

This observation was substantiated by a series of experiments in which apertures of different sizes were used. The diffusion effect, measured by the enlargement of the radiated region, was more pronounced the smaller the aperture.

### *The Action of the Radiations on Opsonin.*

The experiment has been described in which citrated blood was exposed to the action of the emanation in a closed glass bulb and the degree of hæmolysis observed from time to time. Simultaneously with these observations the opsonic content of the blood was compared with that of the control.

The usual procedure in estimations of opsonin was adopted and an emulsion of *Staphylococcus aureus* was used in every case. The first estimation, which was made 19 hours after exposure of the blood to the emanation had begun, gave evidence of a reduction of the opsonic content. The leucocytes used had, however, been subject to the radiations, and were degenerated. In consequence of this action the radiated blood was centrifugalised and the opsonin content of the supernatant fluid was determined with freshly washed leucocytes.

After 44 hours' exposure the following result was obtained :—

| | | | | |
|---|---|---|---|---|
| Control fluid gave ...... | 372 | micro-organisms in | 60 | leucocytes. |
| Experimental fluid gave | 34 | „ | 60 | „ |

The effect, then, of long exposure is apparently a marked reduction in the opsonin.

According to some experiments by H. Reiter,* in which emanation was mixed with blood for short periods of time, an *increase* of the phagocytic power of blood cells was observed, for to quote from this author—"Soweit nach den Versuchen *in vitro* zu erteilen ist, scheint die Emanation die phagocytäre Tätigkeit der Blutzellen anzuregen, in einzellen Fällen bis zu 30°/₀."

The experiment was varied, as, during the previous exposure, the serum was the recipient of products of radiated corpuscles, the action of which might possibly injuriously affect phagocytosis. Some citrated blood was centrifugalised and about 1 c.c. of the supernatant fluid was exposed to the emanation and examined on two occasions with the following results :—

| Time of exposure. | | | | | |
|---|---|---|---|---|---|
| 19 hours | Control fluid gave ...... | 495 | micro-organisms in | 160 | leucocytes, |
| | Experimental fluid gave | 84 | „ | 160 | „ |
| 42 „ | Control fluid gave ...... | 388 | „ | 100 | „ |
| | Experimental fluid gave | 57 | „ | 100 | „ |

In order to test whether there were substances formed in the radiated fluid which might be inhibitory to phagocytosis, further observations were made with the two plasmas of the last experiment. The procedure is indicated as follows :—

Experimental plasma + Control plasma + Micro-organisms + Washed leucocytes.—Film I.
1 volume | 1 volume | 1 volume | 2 volumes.
Saline solution + Control plasma + Micro-organisms + Washed leucocytes.—Film II.

---

* H. Reiter, 'Zentralblatt für Röntgenstrahlen, Radium, etc.,' 1910, p. 243.

A count of the two films which were prepared gave the following results :—

Film I gave 441 micro-organisms in 150 leucocytes.
Film II „ 541 „ 150 „

It is clear that if the experimental plasma had contained substances inhibitory to phagocytosis, their influence should still be manifest when added to the control plasma. The difference between the counts of the two films, amounting as it did to only 20 per cent., shows that the large reduction previously observed with the radiated serum is mainly to be attributed to a reduction in the opsonin normally present.

The effective agent is here again the $\alpha$-particle. By excluding this type of radiation and exposing serum to the $\beta$- and $\gamma$-rays, no appreciable alteration in its opsonic content was obtained with quantities of the order 5 to 10 mgrm. $RaBr_2$, and for exposures lasting about forty hours.

A series of observations was made by taking a measured volume of serum (12·5 cu. mm.) spread over an area of 1·5 sq. cm. on a thin mica sheet and exposing it to the $\alpha$-radiation from one of the capsules. A fresh sample of serum was used for each exposure, as, owing to the very limited penetration of the fluid by the $\alpha$-rays, only just sufficient for an opsonic determination was radiated. After the serum had been radiated for any selected interval, its opsonic content was compared with that of the control.

The results obtained are given in Table IV and shown graphically in fig. 6 :—

Table IV.

| Time of radiation. | Percentage of opsonin in radiated serum. | Number of micro-organisms in 100 leucocytes. | |
|---|---|---|---|
| | | Control. | Experiment. |
| h. m. | | | |
| 2 40 | 82 | 620 | 506 |
| 5 15 | 58 | 620 | 360 |
| 10 30 | 59 | 386 | 228 |
| 14 0 | 31 42 per cent.* | 430 | 182 |
| 18 20 | 35 47 „ * | 507 | 244 |
| 20 15 | 24 | 210 | 50 |
| 36 45 | 14 | 383 | 53 |
| 45 0 | 9 | 681 | 64 |

* A smaller quantity of Ra was used in these cases, the numbers on reduction give 31 and 35 per cent.

The general character of the curve is of the simple exponential type, but owing to the possibility that the method of estimating the amount of

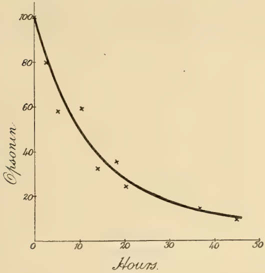

FIG. 6.

opsonin is not strictly quantitative, it cannot be asserted that the destruction of opsonin rigorously follows an exponential law.

### *The Action of the Radiations on Complement.*

A similar series of observations was made with hæmolytic complement. For this purpose a thin film of serum was exposed to the α-radiation from one of the capsules in the manner already described. The complement in the radiated serum was then compared with that contained in the control.

In order to obtain a quantitative estimate of the complement, a method similar to that described by Dr. Emery* has been used.

Briefly, the method consists in procuring a 20-per-cent. suspension of fully sensitized red blood corpuscles, obtained by adding to 1 volume of washed corpuscles, 4 volumes of a strong immune serum. To 4 volumes of this suspension is added 1 volume of the serum, the complement in which is being tested. After being kept at 37° C. till hæmolysis is complete, the liquid is centrifugalised and the amount of free hæmoglobin in a constant volume (76 cu. mm.) of the supernatant fluid tested by means of a Sahli hæmometer.

This instrument was calibrated by adding to 4 volumes of sensitized red corpuscles 1 volume of serum of varying dilutions. The amount of free hæmoglobin as read from the scale of the instrument was found, within the limits of the experimental error, to be proportional to the strength of the serum added, and therefore to the amount of complement present, as may be seen from fig. 7. For hæmometer readings below 20 accuracy is not claimed.

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* Emery, 'Lancet,' 1911, p. 490.

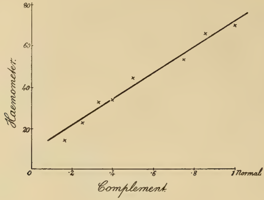

Fig. 7.

The volume of serum exposed to the radiation in the opsonin experiments was 12·5 cu. mm., but this being an inconveniently small quantity in the complement estimations, the volume was increased to 30·5 cu. mm. After an exposure of any desired interval the amount of complement in the radiated serum was compared with that in the control. The results may be seen from Table V and fig. 8.

Table V.

| Time of exposure. | | Percentage of complement remaining. |
|---|---|---|
| h. | m. | |
| 13 | 15 | 92 |
| 17 | 30 | 82·5 |
| 22 | 15 | 76·6 |
| 28 | 0 | 86 |
| 36 | 25 | 64 |
| 41 | 15 | 48 |
| 44 | 35 | 46·8 |
| 46 | 30 | 52·9 |
| 54 | 15 | 15 estimated |
| 66 | 30 | 10 ,, |

A comparison between this curve and that in fig. 6 shows a striking dissimilarity in the reductions of opsonin and of complement in serum when subject to α-radiation; but the two curves are not quantitatively comparable, owing to the difference in the volume of serum radiated in the two cases. For this reason three determinations of the reduction in opsonin were made in which the same volume of serum was radiated as in the complement experiments. These three observations are indicated by the thin line curve, fig. 8. The type of the curve is identical with that previously obtained, the only difference being a diminished rate of reduction of opsonin, as was to be anticipated.

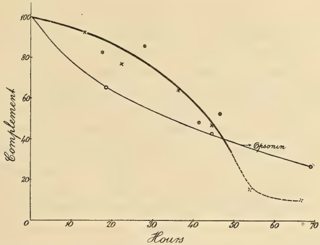

Fig. 8.—The crosses (×) on the complement curve indicate values obtained with the use of sensitized sheep's corpuscles, the circles (o) those obtained with sensitized human corpuscles.

Inspection of the complement curve shows that the reduction is slow at first, the rate gradually increasing with time, as may be seen from the convexity of the curve to the time axis.

The experimental and control sera were, except for the radiation, kept under identical conditions at room temperature. The spontaneous disappearance of complement is therefore eliminated as a disturbing factor, by a comparison between the two sera. It should be pointed out, however, that fresh serum was used for every experiment, because it was found that the complement in serum which has been kept for some days at 0° C. was more affected by the radiation than fresh serum.

The general character of the two curves (fig. 8) indicates the separate identity of opsonin and complement.

The complement curve suggests either that this substance becomes more unstable under the action of the radiation, or that its amount is in some way dependent upon some other substance present in serum.

The small initial reduction in complement could, on the latter supposition, be explained if this substance under the action of the radiation were eventually reduced to complement; then, despite the simultaneous reduction in the latter, a supply would be provided owing to the breaking down of the former.

Our present methods need elaboration before this question can be settled.

### *Summary of Conclusions.*

1. Red blood corpuscles are hæmolysed by the action of $\alpha$-rays, and oxy-hæmoglobin is converted into met-hæmoglobin.

2. Leucocytes undergo marked degenerative changes when subjected to $\alpha$-rays. During the process of clotting, leucocytes appear to move away from an $\alpha$-radiated region. This movement has been attributed to changes found to occur in the surface tension of blood serum when radiated.

3. The specific properties of opsonin and hæmolytic complement are lost when serum is exposed to $\alpha$-rays. The progressive changes caused by these rays indicate the separate identity of opsonin and complement.

4. The $\beta$- and $\gamma$-rays have yielded negative results in analogous experiments.

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## *On a New Method of Estimating the Aperture of Stomata.*

By Francis Darwin, F.R.S., and D. F. M. Pertz.

(Received June 15,—Read June 29, 1911.)

CONTENTS.

PAGE

§ 1. Method ........ 136

§ 2. Difficulties ........ 139

§ 3. Comparison with other Methods ........ 139

§ 4. Stomatal Aperture and Transpiration ........ 141

§ 5. Light and Darkness ........ 143

§ 6. Withering ........ 149

§ 7. Conclusion ........ 153

### § 1. *Method.*

It is usually assumed that transpiration is regulated by two principal factors: (1) the relative humidity of the air, and (2) the degree of aperture of the stomata. Neither of these assumptions has been experimentally proved, though both of them are necessarily true, but it must be remembered that the factors referred to are not necessarily the only ones that govern the phenomena.

The experiments hitherto made on (1) the effect of relative humidity are vitiated by want of precise knowledge as to the stomatal aperture during the course of the enquiry.*

Hitherto it has also been found difficult to test assumption (2), *i.e.*, that transpiration is a function of stomatal aperture, because we had no really trustworthy method of estimating that aperture in the living leaf. Stahl's cobalt method† and the horn hygroscope‡ are not free from Lloyd's§ objection to them—that they indicate variations in the yield of water-vapour which need not necessarily be dependent on changes in stomatal aperture. The point we are discussing is the subject matter of Lloyd's book on stomata. His method of observation is to strip the epidermis from the living leaf and plunge it at once into absolute alcohol. He asserts that specimens so prepared exhibit under the microscope the condition of the stoma as it was in life. The result of a series of careful measurements, compared with records of transpiration, is to convince Lloyd that the aperture of the stoma is *not the dominant factor*, and that transpiration is, on the contrary, regulated by some unknown properties of the plant. He does not, I think, tell us what he suspects these unknown properties to be, but it is not difficult to imagine internal interference with the loss of water.

Although we recognise the value of Lloyd's work, we are not convinced by it; we believe that a much more intimate knowledge of the *living* stoma and its movements would be necessary to prove his contention|| that "the regulatory function is almost *nil*." With a view to testing the question we have designed an instrument which we propose to call a porometer.¶ The idea is to estimate changes in the stomata by recording the change in the velocity of a current of air drawn through them in the living leaf. The construction is shown in the following figure in a diagrammatic form.

A glass chamber (C) bearing a broad flange is cemented to the stomatal surface of a leaf (L). A rubber tube connects C with a tube (T), one limb of which is graduated and dips into the vessel of water (V). The other limb ends in a tube controlled by a clamp (M). By applying suction (as indicated by the arrow) and then closing the clamp M, a column of water is raised to A.

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* We hope to publish before long a method of demonstrating the dependence of transpiration on relative humidity, of which some account was given at the Sheffield meeting of the British Association in 1910, when some of the experiments given in the present paper were also described.

† Stahl ('94). (See Bibliography, p. 154, *infra*.).

‡ F. Darwin ('98).

§ Lloyd ('08).

|| Lloyd ('08), p. 45; elsewhere (pp. 35 and 44) he allows that the extreme limits of transpiration are fixed by the stomata.

¶ It is on the same general principle as N. C. J. Müller's apparatus, which never came into use owing to its cumbrous make. See Müller, N. ('73).

The pressure within the chamber C being thus diminished, air is sucked through the stomata into the chamber, and the water column falls to its original level B. By again applying suction, the column is again raised, and the observation can be repeated as often as may be necessary. The time in which the column falls, say from A to B, is recorded. We thus get a series of readings of the rate of flow at the mean pressure $\frac{1}{2}(A+B)$. The mean is generally 20 cm. of water, the fall of the meniscus being timed either from 23–17 cm., or 22–18 or 21–19, as may be most convenient. The tube is commonly of such a bore that 1 cm. in length = 0·1 c.c.

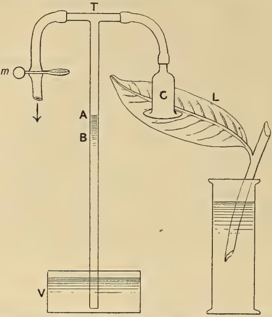

Fig. 1.—Porometer attached to a leaf, see the text for explanations.

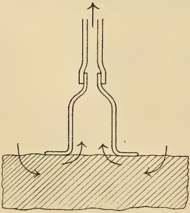

Fig. 2.

Fig. 2 shows a chamber fixed to an enormously exaggerated leaf; the arrows show the air entering the leaf outside and emerging from the leaf inside the chamber.

It is obvious, if successive readings are made at a known mean pressure, that a diminution in the aperture of the stomata will give a slower fall of the water column (fig. 1) from A to B. And as a matter of fact it is found that such diminution of flow is produced by the well-known causes of stomatal closure, such as darkness or faint illumination, withering, poisons, etc.

### § 2. *Difficulties.*

The chief difficulty encountered was to find a method of fixing the chamber to the leaf which should be non-injurious and should make an air-tight joint. After many trials we conclude that ordinary glue is the best medium: it adheres well both to the leaf and to the glass flange, and is not injurious. The best way is to let the glue get fairly cool (say 30° C.), and paint it thickly on to the flange of the chamber, which is then gently pressed on to the stomatal surface of the (inverted) leaf and clamped in that position, the leaf being supported on a horizontal glass plate (not shown in fig. 1). Another method is to cut out a washer (*i.e.*, a perforated disc) from a layer of 20–25 per cent. gelatine about a centimetre in thickness, and to press the chamber firmly down on the washer, and clamp it in that position. Here, again, the leaf has the stomatal side upwards, and is supported by a horizontal glass plate. This method is best for tough leaves, *e.g.*, *Ficus elastica*, *P. laurocerasus*, ivy (*H. helix*), etc., which do not appear to be injured in spite of being compressed between the gelatine and the supporting glass plate. With care, however, delicate leaves may safely be treated in the same way. In the earlier experiments gelatine with a percentage of glycerine was used; this, however, is injurious. The same may be said of vaseline and other greasy substances which were employed at an early stage of the inquiry.

### § 3. *The Porometer compared with other methods, i.e., the Cobalt Test, the Horn Hygroscope, and Lloyd's Microscopic Method.*

The porometric method is a direct one, and must therefore be classed with the microscopic method, since both are absolutely independent of transpiration,* whereas the two hygroscopic methods are indirect, and alterations in stomatal aperture cannot be inferred with certainty from the observations in question.

The porometer shares with the last-named methods the great advantage of being continuous, that is to say, it allows of prolonged observation of a given leaf. Lloyd's method entails the sacrifice of a leaf for each observation. Moreover, his measurements are not actual observations on living tissue, whereas the porometer and the hygroscopic methods have this merit.

A striking point about the porometer is its great range. Thus the rate of air-flow in an illuminated leaf may be as much as 400 times as rapid as the flow through the same leaf in darkness. The porometer, being more delicate than the hygroscopic methods, confirms the observations made some years ago* on withering leaves, in which the stomata were apparently open long after the leaf ceased to react to the horn hygroscope.

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* All that is here meant is that the flow of air through the stomata is not in any way influenced by the amount of water vapour diffusing through the same openings.

The great advantage of Lloyd's microscopic method is that it gives (assuming it to be trustworthy) absolute quantities, *i.e.*, it gives the actual size of the stomatal pore. The porometer only gives relative sizes. Lloyd's method suffers from the fact that, on a given leaf at a given moment, stomata are found varying from 10 to 1 units in diameter. And, since it is impossible to give unlimited time to each reading, it follows that his determinations of stomatal sizes are rather rough. The porometer, on the other hand, automatically strikes an average of many hundred stomata at each reading, and this we believe to be a great advantage over the microscopic method.

A cognate fact is that, on a given branch at a given time, the different leaves may have stomata in very different conditions as to aperture. The following figures illustrate the differences observed in the laurel:—

Experiment 78.—*P. laurocerasus.* October 18, 1910.

The leaves were numbered from the apex downwards, and were all of the current year except Nos. 12 and 13, which were unhealthy-looking leaves of 1909. The fall of the column was timed for 4 cm., *i.e.*, 22–18 cm.

| Leaf No. .......................... | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|
| Times in seconds ............ | 6·9 | 15·0 | 14·9 | 5·6 | 14·4 | 6·3 | 19·5 | 140·0 | 78·0 |

The observations lasted from 10.50 to 11.40 A.M. It will be seen that the rates of flow of the 1910 leaves varied from 5·6 to 19·5, or from 1 to 3·5. The stomata on the old leaves, Nos. 12 and 13, are considerably more closed. If this fact is true for other plants (and we have reason to believe it is so), it is clear that any comparison between transpiration and the aperture of the stomata, as estimated by the rate of flow through a *single leaf*, is not necessarily trustworthy, because the transpiration of a branch must depend on the average aperture of the stomata on a number of leaves, while the rate of flow is dependent on the behaviour of a single leaf. From this point of view Lloyd's method must be commended, it being part of his technique that many leaves should be examined.†

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* F. Darwin ('98), p. 547.

† Lloyd ('08), p. 23.

### § 4. *Stomatal Aperture in Relation to Transpiration.*

The object of the present paper is to give an account of the new method, and to illustrate its applicability to some of the problems of stomatal movement. Nevertheless, we propose to give a single instance of the resemblance which exists between variations in the transpiration rate, on the one hand, and the variation in the size of the stomata as indicated by the porometer. This resemblance, which forms the subject of a future paper, can only be established by the average result of a considerable number of experiments,* partly, no doubt, because of the irregularity in the stomatal behaviour of individual leaves, but probably for other reasons as well. The following experiment is merely intended to show that the correspondence between the two curves may be fairly close. *But variations from this degree of parallelism are both common and great.*

The curve S in fig. 3 is constructed not from the rate of air-flow through the leaf, but from the *square root* of the rate ($\sqrt{R}$). Our experience is that a curve so constructed follows the transpiration curve more closely than curves built from any other function of the rate of flow.

We hope elsewhere to give an account of the theoretical considerations bearing on the problem, and for these we are indebted to the kindness of Sir Joseph Larmor. It is not certain that we shall ever be able to deduce the size of the stomata from the readings of the porometer; but it seems well to make use of the square root of the air-flow in recording our results, and to do so even in the experiments in which the relation between stomatal aperture and transpiration is not directly investigated. And this rule has been generally followed.

Experiment 75; October 10, 1910.—Laurel, *P. laurocerasus.* Temp. 15–16°. $\psi$ (*i.e.*, psychrometer), 70–79 per cent.

Branch of laurel having 17 of the current year's leaves, cut about 9.30 A.M., surface of stem vaselined and fitted to a postometer and a porometer.

The plant was exposed to bright diffused light when not in the dark room.

The postometer readings have been roughly corrected to a constant degree of relative humidity.

---

* In our judgment the experiments already made, but not published, do establish a relation between stomatal aperture and transpiration.

Experiment 75.

| Time. | Postometer. | Rate, corrected. | Porometer. | Rate. | √Rate. |
|---|---|---|---|---|---|
| A.M. | Seconds. | | Seconds. | | |
| 10.57 | 50 | 21·9 | | | |
| 11.5 | | | 8·7 | 1149 | 33·9 |
| 13 | | | 7·9 | 1266 | 35·6 |
| 19 | 48 | 22·4 | | | |
| 20 | | | 7·2 | 1389 | 37·3 |
| 33 | | | 7 | 1429 | 37·8 |
| 39 | *Dark* | | | | |
| 43 | | | 6·8 | 1471 | 38·4 |
| 45 | 42 | 24·7 | | | |
| 47 | | | 7 | 1429 | 37·8 |
| 53 | 52 | 19·2 | 8 | 1250 | 35·4 |
| 57 | 57·5 | 17·4 | | | |
| 58 | | | 8·5 | 1176 | 34·3 |
| P.M. | | | | | |
| 12.4 | 67·5 | 14·8 | | | |
| 8 | | | 12 | 833·3 | 28·9 |
| 11 | 79 | 12·7 | | | |
| 18 | 82 | 12·2 | | | |
| 26 | | | 15 | 667·7 | 25·8 |
| 30 | 96 | 10·4 | | | |
| 44 | | | 20 | 500 | 22·4 |
| 47 | 110 | 9·1 | | | |
| 56 | | | 25 | 400 | 20 |
| 58 | 117 | 8·5 | | | |
| 1.49 | | | 66 | 151·5 | 12·3 |
| 57 | 159 | 6·3 | | | |
| 2.0 | | | 76·8 | 130·2 | 11·4 |
| 3 | 174 | 5·7 | | | |
| 5 | *Light* | | | | |
| 11 | | | 81·3 | 123 | 11·1 |
| 12 | 144 | 5·9 | | | |
| 19 | 128·6 | 6·7 | | | |
| 32 | 56·8 | 7·3 | | | |
| 46 | | | 33·5 | 298·5 | 17·3 |
| 47 | 98·6 | 8·4 | | | |
| 59 | | | 25·4 | 393·7 | 19·8 |
| 3.2 | 115 | 7·0 | | | |
| 12 | | | 27·8 | 359·7 | 19·0 |
| 30 | | | 45 | 222·2 | 14·9 |
| 35 | | | 53 | 188·7 | 13·7 |
| 46 | | | 87·5 | 114·3 | 10·7 |
| 49 | 290 | 3·0 | | | |
| 53 | | | 123 | 81·3 | 9·0 |

The most striking departure of the stomatal curve S (the square root of rate of flow) from the transpiration curve T is between 2 and 4 P.M. This will be seen if the salient points in the two curves are reduced to a common scale by assuming that, at one of these points (*e.g.*, the end of the dark period), both transpiration and √ flow are equal to 1, and calculating the other points on that basis.

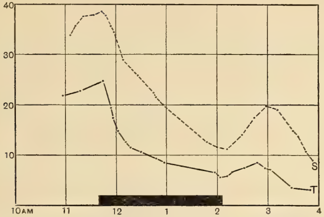

FIG. 3.—Experiment 75. Curve S is constructed from the square root of the rate of air-flow. T represents transpiration. The black bar gives the period of darkness.

It is evident that the agreement of the columns S and T is only a rough one, and that in some points the differences are great. But if we replace

| Times (roughly). | S. √Flow. | T. Transpiration. |
|---|---|---|
| A.M. | | |
| 11.5 | 3·1 | 3·7 |
| 45 | 3·5 | 4·0 |
| P.M. | | |
| 2.10 | 1·0 | 1·0 |
| 48—59 | 1·8 | 1·3 |
| 3.50—55 | 0·8 | 0·5 |

the √ flow by the actual rate of flow, we get a series of figures in which the discrepancy is, on the whole, much greater between transpiration and the porometer record.

| Flow. | Transpiration. |
|---|---|
| 9·6 | 3·7 |
| 12·3 | 4·0 |
| 1·0 | 1·0 |
| 3·2 | 1·3 |
| 0·6 | 0·5 |

## § 5. *Light and Darkness.*

In the following experiments, *light* means exposure, at a north or east window, to diffused light, the plant being placed as near the glass as may be. *Darkness* means that the experiment is continued in a dark room.

The period of darkness is indicated on the diagrams by a black bar on the time scale.

In the tables, R stands for the proportional rate of air-flow obtained from the reciprocals of the second column. The fourth column gives the square root of the rate of flow.

Experiment 38; July 8, 1910.—Tropæolum (in a pot). North window. Temp., 15–16°. ψ, 72–75 per cent.*

| | | R. | √R. |
|---|---|---|---|
| A.M. | Seconds. | | |
| 9.52 | 7·0 | 1429 | 37·8 |
| 10.5 | 6·6 | 1515 | 38·9 |
| 46 | 7·0 | 1429 | 37·8 |
| 11.2 | *Darkness* | | |
| 10 | 8·0 | 1250 | 35·4 |
| 33 | 18·0 | 556 | 23·6 |
| 51 | 54·0 | 185 | 13·6 |
| P.M. | | | |
| 12.20 | 152·0 | 65·8 | 8·1 |
| 33 | 189·6 | 52·7 | 7·3 |
| 1.42 | 336·0 | 29·8 | 5·5 |
| 3.8 | 583·2 | 17·2 | 4·1 |

* The symbol ψ is used for the relative humidity of the air.

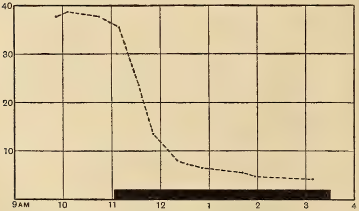

FIG. 4.—Experiment 38. Partial closure of stomata in darkness.

It will be seen that there is a rapid fall in the curve at about 5 minutes after the beginning of the dark period, and that the curve gradually flattens until, at 3 P.M., it is approaching steadiness.

Experiment 65; August 4, 1910.—*Helianthus annuus.* Temp. 20–21°.
$\psi$, 58–62 per cent.

A cut leaf in water. In order to get a fairly readable fall of the water column, it was found necessary to use a tube in which 1 cm. = 0·4 c.c. instead of the usual 0·1 tube. The square root only of rate of flow ($\sqrt{R}$) is given.

| | $\sqrt{R}$. | | $\sqrt{R}$. |
|---|---|---|---|
| A.M. | | P.M. | |
| 10.49 | 3·7 | 12.53 | 4·8 |
| 56 | 4·0 | 58 | *Dark* |
| 11.16 | 5·2 | 1.4 | 3·3 |
| 25 | *Dark* | 13 | 2·3 |
| 30 | 4·1 | 26 | 1·9 |
| | | 38 | 1·8 |
| P.M. | | 2.10 | 1·8 |
| 12.6 | 3·2 | 15 | *Light* |
| 15 | *Light* | 25 | 2·2 |
| 22 | 3·4 | 36 | 2·4 |
| 29 | 3·6 | 49 | 2·9 |
| 39 | 4·3 | 3.11 | 3·7 |

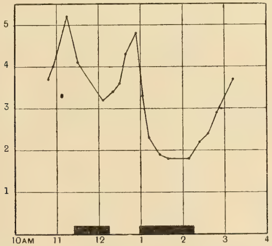

Fig. 5.—Experiment 65. Partial closure and reopening in darkness and light.

It will be seen that from 10.49 to 11.25 A.M., when the first dark period began, the stomata were opening; the exact moment at which the fall in the curve (*i.e.*, the closure of the stomata) began is not shown, because the observations are not numerous enough, but 6 minutes after the beginning of darkness the rapid fall had begun. The first dark period, of about 50 minutes, was not long enough to bring the stomata to rest, but later in the day, between 1 and 2 P.M., half-an-hour's darkness brings the curve to the horizontal. The rapid opening of the stomata at 12.15 and 2.15 P.M. is well shown.

It is well known* that aquatic and marsh plants do not close their stomata in darkness. The following experiment illustrates the fact:—

Experiment 30P.—*Alisma sp.* June 28, 1910. Temp. 17–18°. ψ, 68–71 per cent.

Porometer on lower surface, upper surface painted with vaseline. Fall of column 12–8 cm., *i.e.*, 4 cm., at an average pressure of 10 cm. The experiment began in the dark room with the shutters open to a south light, but no sunlight shone on the leaf. In this instance we have simply given the average number of seconds required for the fall of the water column, because in this way any change in the aperture is more strikingly obvious.

| June 28. | |
|---|---|
| A.M. | Seconds. |
| 10.38—55 | 6·2 |
| 11.0 | *Dark* |
| 13—56 | 6·5 |
| 12 noon | *Light* |
| P.M. | |
| 12.2—50 | 6·6 |
| 51 | *Dark* |
| 3.45—46 | 6·8 |
| June 29. | |
| A.M. | |
| 10.13—14 | 7 |
| 15 | *Light* |
| 16—50 | 6·7 |

In other experiments the result is not so simple.

Experiment 24P.—*Alisma sp.* June 23. Conditions as in Experiment 30P.

| June 23. | | June 24. | |
|---|---|---|---|
| A.M. | Seconds. | A.M. | Seconds. |
| 11.0 | 6 | | *Dark* |
| 19 | 10 | 9.43 | 26 |
| 20 | *Dark* | 45 | 27 |
| 32 | 10 | 56 | 27 |
| 56 | 13 | | *Light* |
| | | 58 | 24 |
| P.M. | | 10.7 | 27 |
| 12.13 | 17 | 11.3 | 23 |
| 32 | 22 | 39 | 24·5 |
| 32·5 | *Light* | 40 | *Dark* |
| 34 | 23 | | |
| 43 | 21 | P.M. | |
| 55 | 21 | 12.2 | 25·5 |
| 1.8 | 16 | 4 | 24·5 |
| 15 | 20 | 4.28 | 44 |
| 2.4 | 26 | 30 | 45·5 |
| 3.31 | 41 | | |
| | *Dark* | | |

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* F. Darwin ('98), p. 579, where references to the literature are given.

Thus on June 23 the rate of air-flow changed considerably in the day, becoming slower on the whole, but not showing any marked effect of light or darkness. On the following day there is irregularity which is not clearly connected with changes in illumination.

It was formerly found* that the stomata of *Caltha palustris* close in darkness. This, however, is not always the case; no closure could be detected in a porometer experiment of June 25, 1910. Further work is needed on this species.

*Nocturnal Closure.*—The following two experiments illustrate the closure and opening of stomata when exposed to diurnal change of illumination. The experimental plants *Nicotiana glauca* (Experiment 67) and *P. laurocerasus* (Experiment 68) were in flower-pots and placed close together in a greenhouse on the roof of the laboratory. The sky was cloudy, except between 5 and 6.30 P.M., when there was occasional faint sunshine. The temperature (T) and relative moisture ($\psi$) of the air are given under Experiment 67. Only the square root of the air flow ($\sqrt{R}$) is given.

Experiment 67; August 9, 1910.—*N. glauca.*

| | $\sqrt{R}$. | T. | $\psi$. | Remarks. |
|---|---|---|---|---|
| P.M. | | | | |
| 2.22 | 30·4 | | | |
| 54 | | 20·3 | 72 | |
| 3.6 | 33·7 | | | |
| 56 | | 20·2 | 70 | |
| 4.24 | 26·9 | | | |
| 5.5 | | | | Faint sun for about ¼ hour. |
| 12 | 25·2 | 20·4 | 74 | |
| 6.16 | 26 | | | Sun obliquely on leaf. |
| 45 | 25·7 | | | |
| 7.47 | 3·7 | | | |
| 59 | 2·7 | 17·0 | 84 | |
| 8.12 | 2·3 | | | |
| 27 | 2·1 | | | |
| 31 | 2·0 | | | |
| 54 | | 16·0 | 87 | |
| 9.9 | 1·9 | | | |
| 10.27 | | 15·2 | 91 | |
| 29 | 2·1 | | | |
| 37 | | 14·4 | 91 | |
| A.M. | | | | |
| 12.10 | 2·8 | | | |
| 1.58 | 3·7 | 13·5 | 91 | |
| 3.30 | 4·5 | 12·8 | 89 | |
| 39 | 4·6 | | | |
| 51 | | | | Notes easily legible; eastern sky red. |
| 4.45 | 6·1 | 12·4 | 90 | |
| 5.55 | | 13·0 | 90 | Light clouds, no sun on plant. |
| 6.5 | 18·3 | | | |
| 7.37 | 34·7 | 15·4 | 85 | |
| 9.1 | 43·4 | 19·2 | 72 | Bright: not sunshine. |

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* F. Darwin ('98), p. 580.

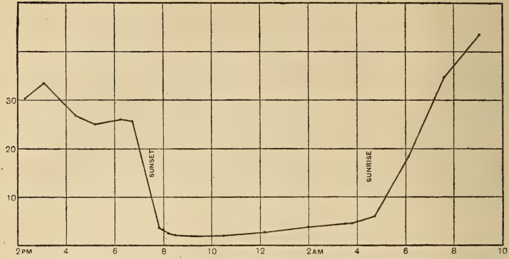

Fig. 6.—Experiment 67. Diurnal behaviour of the stomata of *Nicotiana.*

Experiment 68; August 9, 1910.—Laurel (*P. laurocerasus*). Conditions as in Experiment 67.

| | √R. | | √R. |
|---|---|---|---|
| P.M. | | A.M. | |
| 3.7 | 39·8 | 12.12 | 10·7 |
| 55 | 45·6 | 2.6 | 11·4 |
| 4.56 | 42·6 | 3.37 | 10·9 |
| 5.11 | 38·4 | 4.49 | 14·4 |
| 6.22 | 30·9 | 6.3 | 28·6 |
| 46 | 28·3 | 7.42 | 43·4 |
| 7.45 | 18·1 | 9.5 | 44·7 |
| 8.7 | 9·1 | | |
| 57 | 4·7 | | |
| 9.17 | 3·8 | | |
| 10.32 | 5·8 | | |

*Note.*—T and $\psi$, etc., as in Experiment 67.

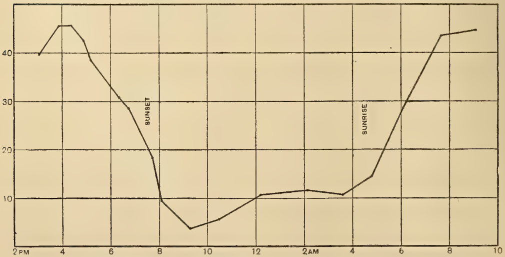

Fig. 7.—Experiment 68. Diurnal behaviour of the stomata of *P. laurocerasus.*

It will be seen that with *N. glauca* (fig. 6) there is a sudden fall in the curve about sunset. Owing to the fact that no readings were taken between 6.45 and 7.47, it is impossible to know at what hour the fall began. In the observations (F. Darwin ('98), p. 595) made with the horn hygroscope on Tropæolum and Pelargonium, there is a similar fact, *i.e.*, a gradual fall during the afternoon and a sudden fall about sunset. But in Experiment 67 the stomata had nearly reached their utmost degree of closure 24 minutes after sunset, whereas in Pelargonium this was clearly not the case, and probably not so in Tropæolum. On the other hand, in Narcissus (*op. cit.*, p. 589) the horn hygroscope was at zero 38 minutes before sunset.

In the observations on the laurel in the present paper (fig. 7), the curve falls from about 4 P.M. to 9.17, no very great effect of sunset being visible.

The most interesting fact observable in figs. 6 and 7 is that the stomata begin to open long before sunrise; in fact, almost as soon as they have reached the maximum of closure. This is especially striking in fig. 7, but is perfectly clear with *N. glauca*, fig. 6. Further work will show whether the phenomenon is a general one.

In both plants a sudden opening of the stomata occurs about sunrise.

There can be little doubt that the opening of the stomata during the early hours of the night is due to periodicity: Lloyd ('08, p. 37) remarks under the heading of "normal daily periodicity" that there seems "to be a tendency for stomata to open a little during the night." He shows (p. 67) also the existence of a rise in transpiration rate during the night and early morning in complete darkness, but (p. 73) he speaks doubtfully as to the connections of this fact with increased stomatal aperture. The whole question of periodicity requires re-investigation with the porometer.

### § 6. *The Effect of Withering.*

In two previous papers* the curious fact has been recorded that the closure of the stomata, produced by depriving a leaf of water-supply, is often preceded by increased transpiration, which was assumed to be due to opening of the stomata and was accordingly described as the "temporary opening."

Lloyd† has investigated the phenomenon in question and concludes that it does not occur. Whether this is due to his relying on the microscopic method or to the fact that he experimented on other plants we cannot say.

The following experiments show very clearly that, at any rate in certain plants, a striking temporary opening of the stomata occurs:—

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* F. Darwin ('98), p. 548, and ('04), p. 89.

† Lloyd ('08), p. 81.

Experiment 41; July 12, 1910.—*Nicotiana glauca* (in pot).

The chamber was fixed to the leaf by plasticine, a method subsequently abandoned because it appeared to injure the leaves. Diffused light.

The rate of flow (R) is given in full, the square root of R is only given for the salient points. The actual observations, *i.e.*, the number of seconds in which the column fell through 6 cm., are given for the extreme points.

| | | R (reciprocals). | √R. |
|---|---|---|---|
| A.M. | Seconds. | | |
| 11.55 | 20 | 50 | 7·1 |
| P.M. | | | |
| 12.9 | | 57·1 | 7·5 |
| 21 | | 52·6 | |
| 24 | | 51·8 | |
| 25 | | Leaf stalk cut, cut surface not greased | |
| 28 | | 47·8 | 6·9 |
| 30 | | 50 | |
| 33 | | 53·8 | |
| 36 | | 62·5 | |
| 41 | | 80 | |
| 46 | | 120·5 | |
| 53 | 6·4 | 156·3 | 12·5 |
| 1.1 | | 128·2 | |
| 5 | | 108·7 | |
| 10 | | 86·9 | |
| 25 | | 51·5 | 7·2 |
| 2.7 | | 26·9 | |
| 26 | | 18·9 | 4·3 |
| 56 | | 14·1 | |
| 57 | | Vaselined cut stalk | |
| 3.3 | | 12·2 | 3·5 |
| 47 | | 7·4 | 2·7 |
| 4.12 | | 5·9 | 2·4 |
| 6.37 | 295 | 3·4 | 1·8 |

The details of the stomatal change are best seen in the larger curve constructed from R (reciprocals of times and therefore proportional to rate of flow). Within five minutes of the severance of the leaf-stalk the stomata had begun to open with great rapidity; the opening continued for 28 minutes, when it was replaced by rapid closure, which gradually became slower, the curve between 4 and 7 P.M. (not shown in the diagram) being a very flat one.

The temporary effect of cutting off water-supply was to increase the rate R in the proportion 50 : 156 or 1 : 3·1. This is much greater than was ever observed with the horn hygroscope. Thus* with *Campanula vidalii* the rise was from 20 to 33, *i.e.*, as 1 : 1·65, and in Tropæolum from 40 to 60, *i.e.*, as 1 : 1·5.

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* F. Darwin ('98), pp. 549, 550.

But in the smaller curve (fig. 8), constructed from $\sqrt{R}$ (the square root of the rate of flow), the rise in the curve is from 7 to 12·5, *i.e.*, as 1 : 1·8. This, as far as it goes, confirms our view that $\sqrt{R}$ represents the amount of transpiration more closely than does R (the rate of air-flow).

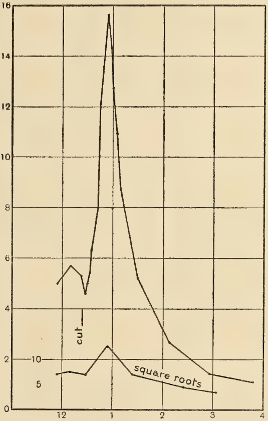

FIG. 8.—Experiment 41. Cutting the leaf-stalk of *Nicotiana glauca*. The small curve constructed from the square root is drawn to twice the scale used for the larger curve.

Experiment 59P; October 25, 1910.—Tropæolum (in pot). At a south window exposed to dull light, no sunshine.

| Time. | | R. | √R. |
|---|---|---|---|
| A.M. | Seconds. | | |
| 10.45 | 15 | 66 | 8·1 |
| 50 | 15 | | |
| 57 | 13 | 76 | 8·7 |
| 11.2 | 11 | | |
| 5 | 10 | 100 | 10 |
| 8 | 9·8 | | |
| 11 | 8·5 | 118 | 10·9 |
| 12 | Cut petiole low down. Greased the cut end. | | |
| 12·5 | 7 | 142 | 11·9 |
| 13 | 6·5 | | |
| 15 | 5·2 | 192 | 13·9 |
| 17 | 5 | | |
| 21 | 3·5 | 286 | 16·9 |
| 22 | 3 | | |
| 11.27 | 2 | 500 | 22·4 |
| 30 | 1·8 | 555 | 23·6 |
| 40 | 2 | 500 | 22·4 |
| 50 | 2 | 500 | 22·4 |
| 12 noon | 1·8 | 555 | 23·6 |
| P.M. | | | |
| 12.8 | 1·1 | 909 | 30·1 |
| 14 | 1·25 | | |
| 18 | 1 | 1000 | 31·6 |
| 43 | 1·4 | 714 | 26·7 |
| 50 | 1·3 | 769 | 27·7 |
| 1.0 | 1·5 | 666 | 25·8 |
| 3.27 | 25·4 | 39 | 6·2 |
| 53 | 48 | 20 | 4·5 |

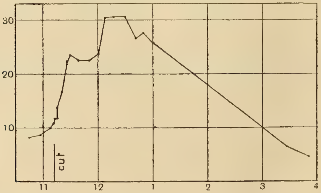

Fig. 9.—Experiment 59P. Cutting the leaf-stalk of Tropæolum. The curve is constructed from the square root of the rate of flow.

The result must be considered as somewhat rough, since when the fall of the water column is so rapid as at 12.18, it is not possible to time it with accuracy. Nevertheless, we may safely assume that the rate of flow increased as from unity to between 8 and 9; this is a much greater opening than occurred with Nicotiana and *a fortiori* than in the experiments with the horn hygroscope. When estimated by $\sqrt{R}$ the opening of the stomata is from 1 to between 2·8 and 3, and this again is greater than anything observed with the horn method.

Another experiment with Tropæolum (October 26, 1910) is here given in an abbreviated form.

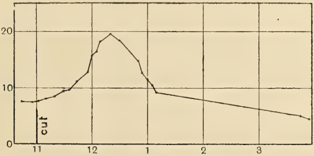

Fig. 10.—Cutting the leaf-stalk of Tropæolum. The curve is constructed from the square root of the rate of flow.

Experiment 60P.—Tropæolum, October 26, 1910.

| | R. | √R. |
|---|---|---|
| A.M. | | |
| 10.46 | 59 | 7·6 |
| 59 | 54 | 7·3 |
| 11.0 | Petiole | cut |
| P.M. | | |
| 12.25 | 384 | 19·5 |
| 3.55 | 20 | 4·5 |

Thus the rate of flow (R) rose as 1 : 6·5, while $\sqrt{R}$ rose as 1 : 2·6.

There is one difference between the results obtained with the porometer and the horn hygroscope, namely, that the opening and subsequent closure are completed much more rapidly with the horn. I imagine that this may be due to the fact that in porometer experiments the act of withering is delayed by the damp air within the chamber.

## § 7. *Conclusion.*

In the foregoing sections our aim has been to give a preliminary account of the porometer and its application to the study of stomata.

The principal merits of the method are (1) that the readings of the instrument are dependent on the aperture of the stomata, and are therefore independent of transpiration. The porometer is accordingly superior to the hygroscopic methods (*e.g.*, Stahl's cobalt test and the horn hygroscope), from which variations in aperture can only be inferred from increased transpiration; (2) the porometer indicates the behaviour of a group of living stomata which can be continuously studied for hours or even days. In this it compares favourably with Lloyd's microscopic method, which, however, has the advantage of giving the actual size of the stomatal pore, instead of merely a series of readings from which changes in the size in the stoma can be inferred.

As a test of the porometer we have selected two of the principal factors that influence stomatal aperture, namely, illumination and water supply; we have shown that the known effects of these agencies are well demonstrated by our method.

In the case of leaves severed from the plant we have confirmed a statement made by one of us, viz., that the first effect of withering is the opening of the stomata, which is, however, followed by closure.

The section on the causal relation between stomatal aperture and transpiration is a single illustration of the conclusion, which we hope to justify when the results of a considerable series of experiments already made are published.

We desire to thank Mr. F. F. Blackman for many useful suggestions made to us in the course of our research.

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# *The Experimental Transmission of Goitre from Man to Animals.*

By Robert McCarrison, M.D., M.R.C.P. (Lond.), Captain, Indian Medical Service; Agency Surgeon, Gilgit, Kashmere.

(Communicated by Major Ronald Ross, C.B., F.R.S. Received May 3,—Read June 1, 1911.)

[Plate 1*.]

Experiments had repeatedly been carried out on dogs to test the assumption that goitre could be conveyed from man to animals by fæcal infection of the water supply, but with negative results. In the present experiments female goats were employed. The drinking water supplied to these goats was fouled by passing through a specially constructed box, which contained sterilised soil mixed with the fæces of goitrous individuals. In the case of one batch of six goats, only this water was consumed. In the case of another batch of seven goats the box above referred to contained, in addition to the sterilised soil and fæces, 500 earthworms. These were added on the assumption that they might act as intermediate hosts to the infecting agent of the disease. The goats consumed this highly polluted water for 64 days, from October 13 to December 15, 1910. The results observed were (1) a loss of weight, due doubtless to confinement in a small hut for the 64 days of the experiment; (2) that many of them suffered from diarrhœa; and (3) that 50 per cent. of the animals showed enlargements of the thyroid gland, most marked on the right side. The thyroids of three control goats showed no alteration in size.

The enlargement of the thyroid was observed to fluctuate in size considerably, a fact which had previously been noted in the case of experimentally produced goitre in man. The average weight of the normal thyroid of the goat in Gilgit is 1/10,000 part of the body weight. The enlarged glands of the goats in the experiment were found to weigh from 1/4,272 to 1/7,000 part of the body weight. In both batches drinking fouled water the results observed were the same.

Microscopical examination of the enlarged organs showed varying degrees of dilatation of the vesicles, scarcity or almost complete disappearance of the masses of cells lying between the vesicles, while no alterations were observed to have taken place in the connective tissue stroma of the enlarged glands. The hypertrophy was due wholly to distension of the vesicle with colloid, and the formation of new vesicles from the intravesicular masses of cells. It is concluded that—

(1) An hypertrophy of the thyroid gland of goats can be induced by infecting the water supply with the fæces of sufferers from goitre. It is at present impossible to state whether this hypertrophy is due to the action of the infecting agent of goitre, or only to the organic impurity of the water thus contaminated.

(2) Earthworms do not appear to be concerned in the spread of goitre.

(3) The microscopical appearances described are the earliest stages in the formation of parenchymatous goitre.

The microphotographs (Plate 1*) illustrate the appearances seen under a magnification of 100 diameters. Fig. 1 shows the normal appearance of the thyroid gland of a goat. Fig. 2 shows the artificially produced parenchymatous goitre.

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# *The Pathogenic Agent in a Case of Human Trypanosomiasis in Nyasaland.*

By Hugh S. Stannus and Warrington Yorke.

(Communicated by Major R. Ross, F.R.S. Received May 3,—Read June 1, 1911.)

[Plate 2.]

Up to the time of writing, nearly 40 cases of trypanosomiasis have been discovered in Nyasaland, whereas *Glossina palpalis*, notwithstanding much careful searching, has not, as yet, been found in the Protectorate. In view of this fact and also of the observation that the trypanosome derived from a case of Sleeping Sickness contracted in North-East Rhodesia has been shown to present certain peculiarities both morphological* and also regarding its pathogenicity† in experimental animals, it appeared to us desirable to examine in some detail the parasite derived from a case of human trypanosomiasis infected in Nyasaland. The trypanosome to which this paper refers was obtained from the blood of Mr. R., Case 12 in the Nyasaland Sleeping Sickness Diary.

The following is a short summary of the history of this case:—Patient

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* Stephens and Fantham, "On the Peculiar Morphology of a Trypanosome from a Case of Sleeping Sickness and the Possibility of its being a New Species (*T. rhodesiense*)," 'Roy. Soc. Proc.,' 1910, B, vol. 83, p. 28.

† Yorke, W., "On the Pathogenicity of a Trypanosome from a Case of Sleeping Sickness Contracted in Rhodesia," 'Annals of Tropical Medicine,' 1910, vol. 4.

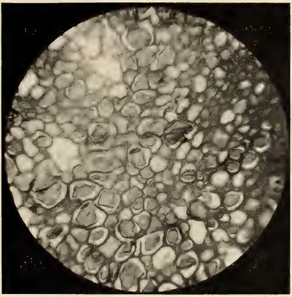

FIG. 1.

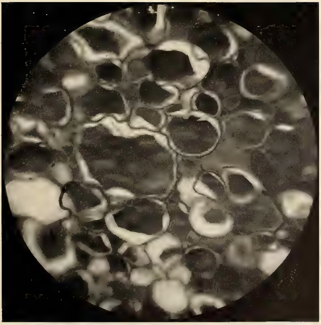

FIG. 2.

entered the Protectorate *viâ* Chinde and Blantyre. Left Blantyre for Angoniland on June 27, 1910. July 1, arrived at Mlanda where he remained four days. July 6, travelled to Mpatso *viâ* Mpungi, and Dedza, thence to Mt. Dzobwe, 40 miles west, and back. July 19, left Mpatso for Diampwi, where he remained until the 23rd. On the 24th he arrived at Mkoma. July 28, reached Mvera, where he remained for about a fortnight. During his stay here he saw a case of trypanosomiasis. The patient was lying in the open air and Mr. R. did not approach within several yards. August 13, left Mvera on a shooting trip and spent the next day at Maganga's village on the lake shore. On August 15 he went to Patsamjoka, where he remained for two days. August 17, arrived at Nsarula on the Lintipe river. Here he was severely bitten by tsetse (species not recognised). August 18, returned to Mvera. August 19, arrived at Kongwe and complained that the bites in the neck were painful; the next day he felt ill. August 23, neck examined by one of his companions, and a "lump" about the size of a shilling, rather light in colour and surrounded by a dark purple ring, was found in the sub-occipital region, where he had been bitten by fly and where he had experienced pain ever since. During the next few days patient complained of severe headache. Temperature 102·5—104° F. Neck, swollen and painful; face, puffy. August 31, blood examined and trypanosomes found in large numbers.

From the above record of the patient's movements whilst in Nyasaland there appears to be little doubt that he was infected in the Dowa sub-district, possibly in the neighbourhood of the Lintipe River, on August 17.

*Treatment.*—For the first five weeks 6 grains of atoxyl were injected intramuscularly on Thursday and Friday of each week. For a second period of five weeks 3 grains of atoxyl were given every third and fourth day. From the 11th to the 18th week soamin (10 grains) was administered on two successive days in every other week, and perchloride of mercury once in the intervening weeks. As the injections of mercury were very painful and were not followed by any improvement, they were discontinued and soamin alone administered in 10-grain doses every Thursday and Friday.

At first the patient's condition steadily improved. On September 24 there was a sudden attack of adenitis, involving the posterior cervical glands of both sides. Since, there has been a gradual but progressive anæmia, with loss of strength and weight. A characteristic rash developed on the 78th day, but disappeared after a few days. It has reappeared on five or six occasions since. There have also been several subsequent attacks of adenitis.

*Temperature.*—Marked periodicity has been a characteristic feature throughout, the temperature rising to 103° to 105° F., and falling again to normal at fairly regular intervals. Although no careful enumeration of trypanosomes was made, yet their numbers were noticed to exhibit a periodicity corresponding to the temperature curve, rises in temperature being associated with increase in the number of trypanosomes in the peripheral blood.

*Morphological Features of the Parasite in the Blood of the Patient.*—Unfortunately, the material at our disposal was rather limited, consisting of a slide of the blood made on August 31, the day on which the disease was first diagnosed, a couple of slides made on November 21, and one on January 4, when the patient passed through Zomba on his way to Chinde. The slide made on August 31 contained numerous trypanosomes, and was sent to the Sleeping Sickness Bureau and examined by Sir David Bruce, who found that the parasite did not differ in any way from the Uganda *T. gambiense.**

In the specimens prepared on November 20 and January 4, trypanosomes were more scanty and the parasite could not be distinguished from *T. gambiense.* The parasite presented the characteristic dimorphism, slender forms with long free flagella, short stumpy forms without free flagella, and intermediate forms being found.

*The Morphology of the Parasite in Animals Experimentally Infected.*—The parasite was also studied in the blood of several experimental animals. An English rabbit, bred in Nyasaland, was infected with the trypanosome by subcutaneous inoculation with a small quantity of the patient's blood, and, subsequently, sub-inoculations were made into a monkey (Cercopithecus) and a goat. The rabbit and monkey both became heavily infected, and exhibited numerous parasites in the peripheral blood, whereas in the goat trypanosomes were only occasionally found in small numbers.

Examination of the parasite in the blood of the rabbit and monkey at once revealed the same morphological peculiarity which was observed by Stephens and Fantham in the trypanosome obtained from a case of Sleeping Sickness contracted in the Luangwa Valley of North-East Rhodesia, *i.e.*, among the stout and stumpy forms, some had the nucleus at the posterior (non-flagellar) end (Plate 2, figs. 5–12 and 14–17). When the parasites were numerous, it was found that these posterior nuclear varieties formed from 1 to 4 per cent. of the total number of trypanosomes present. Posterior nuclear forms were only observed when the blood contained fairly numerous parasites. They measured 17–22 $\mu$ long.

The other parasites found were indistinguishable from *T. gambiense,* and exhibited the usual dimorphism. The cytoplasm of many of the parasites observed in the blood of the monkey was vacuolated in a remarkable manner, sometimes as many as five or six large clear vacuoles were seen in a single trypanosome (figs. 3, 4, 5, 7, and 9). In many of the parasites the cytoplasm contained large, coarse granules. The posterior extremity of many of the parasites—especially those in which the nucleus was situated posteriorly—presented a blunt, "cut away" appearance. Parasites similar to those described by Stephens and Fantham as "snout" forms were likewise observed, but they did not appear to us to be a prominent feature. After finding these posterior nuclear forms in the blood of the rabbit and monkey, we re-examined carefully the slide of the blood of the patient himself, made on August 31, at a time when the parasites were numerous. A prolonged search failed to reveal the presence of any typical posterior nuclear forms, but several dividing forms were seen, in which one of the nuclei was situated close to the blepharoplast (figs. 16 and 17).

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* 'Sleeping Sickness Bulletin,' 1910, vol. 2, No. 21, p. 346.

*Pathogenicity.*—Unfortunately, absence of laboratory animals prevented the investigation of this point. The three animals (rabbit, monkey, and a goat) inoculated with the strain by one of us (H. S. S.) in Nyasaland were all easily infected.

*Rabbit.*—Inoculated subcutaneously with blood from the patient. The temperature rose on the sixth day to 105° F., and parasites were found in the blood in small numbers. The animal died on the 27th day. During the last six days trypanosomes were present in considerable numbers. The symptoms observed were those usually found in rabbits suffering from trypanosomiasis, viz., anæmia, emaciation, œdema of the face and ears, and purulent discharge from the nose and eyes.

*Monkey.*—Inoculated from the rabbit. Parasites found in the blood on the seventh day. During the next week trypanosomes were present in considerable numbers, but later they were scanty. The animal was still alive on the 36th day, but was very anæmic and emaciated. There was distinct auto-agglutination of the red blood cells.

*Goat.*—Inoculated from the rabbit. Trypanosomes found in the peripheral blood on the 15th day and on frequent occasions, but always in small numbers, until the death of the animal, which occurred on the 28th day. The symptoms noted were anæmia, wasting, and œdematous swelling of face. The rapidity of the course which the disease ran in this animal is worthy of remark and is in accordance with the observations of one of us working with the parasite obtained from a case of trypanosomiasis infected in the Luangwa Valley.*

*Conclusions.*—As a result of our observations we are of opinion that the trypanosome in question is not *T. gambiense.* On the other hand this trypanosome resembles very closely *T. rhodesiense*, and is probably identical with it.

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* Yorke, W., *loc. cit.*

The disease was contracted in a district (Dowa sub-district of Angoniland), where *Glossina palpalis* has never been found, but where *Glossina morsitans* is known to exist in large numbers. It appears probable, therefore, that this trypanosome (*T. rhodesiense*) is a distinct species which is capable of transmission by some other agent than *Glossina palpalis*, probably *Glossina morsitans.*

## EXPLANATION OF PLATE 2.

Drawn with Abbé camera lucida, using 2 mm. apochromatic objective and No. 18 compensating ocular (Zeiss). Magnification 2150 diameters.

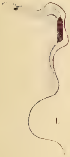
Figures drawn from parasites in the blood of the monkey except when otherwise stated.

Figs. 1—4.—Forms with the nucleus median. Figs. 1 and 2 show line connecting blepharoplast with nucleus ; in figs. 3 and 4 marked vacuolation of cytoplasm is seen.

Figs. 5—12.—Forms in which the nucleus is seen to become gradually more posterior until it lies on a level with the centrosome (fig. 5 from patient's blood, fig. 8 from rabbit's blood).

Fig. 13.—Division form with nucleus median (from patient's blood).

Figs. 14—17.—Division forms with one or both nuclei posterior (figs. 16 and 17 from patient's blood).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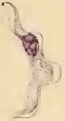
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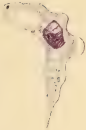
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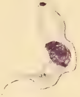
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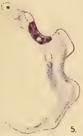
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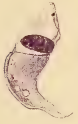
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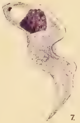
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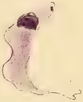
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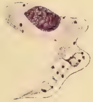
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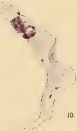
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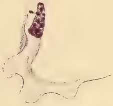
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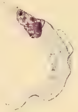
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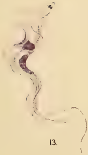
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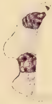
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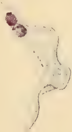
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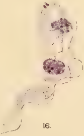
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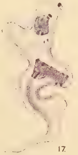
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A.M.B., del. E.Wilson, Cambridge.

# *Note on Developmental Forms of* Trypanosoma brucei (pecaudi) *in the Internal Organs, Axillary Glands and Bone-marrow of the Gerbil* (Gerbillus pygargus).

By George Buchanan, Senior Laboratory Assistant, Wellcome Tropical Research Laboratories, Gordon College, Khartoum.

(Communicated by Col. Sir David Bruce, C.B., F.R.S. Received May 12,—Read June 15, 1911.)

[Plate 3.]

The transmission and maintenance of the trypanosome strains being under my care, ample opportunities were afforded of noting the progress of the disease induced by them in experimental animals and the structural changes in the trypanosomes themselves at varying periods. But it was mainly the cultural work associated with this trypanosome that led to further investigation of these developmental forms and thereby established the observations forming the subject of this note.

On beginning the culture work, inability to recognise if certain forms met with in cultures were either developmental or degenerative phases, suggested a study of the changes which the parasite undergoes, both in the body of the gerbil after death and in citrated blood, and the comparison of these changes with the forms found in Novy and MacNeal and Nicolle's media. With this in view, a gerbil was inoculated with infected blood and chloroformed on the fifth day after injection. Cultures from the heart's blood and smears from the various organs were made, all being examined at regular intervals for comparison. Light was thereby thrown on the point in question, but, in addition to this, attention was drawn to what undoubtedly looked like trypanosome forms in the red blood corpuscles in the spleen. Some of these were identical with those figured by Chagas in his paper on *Schizotrypanum cruzi*, which appeared at the time this work was being conducted.

In order to confirm the observations made, a series of inoculations was carried out in gerbils. In the first of the series, five gerbils were employed, these receiving subcutaneous inoculation in the flanks. In each of the two later series, however, it was found necessary to use eight gerbils at least, the injection being given intraperitoneally as recommended by Chagas. The amount of infected blood in citrate inoculated into each animal was 10 minims, all the eight forming one series receiving the injection at the same time. A gerbil of the series was then chloroformed on each succeeding day, beginning on the first day after injection. Films from the peripheral and heart's blood were taken, and smears from the lung, liver, spleen, bone-marrow and axillary glands were made, these being fixed and stained by various methods.

As both the intra-corpuscular forms and those which possibly represent encysted stages appeared in the spleen on the fifth day in the first gerbil, a careful examination of the films in the first series up to that time was made, but none of the forms seen in the original slide were recognised. In the other series, however, examination of spleen smears from gerbils chloroformed on the sixth and seventh day respectively revealed both of the forms mentioned. These were also usually obtained in subsequent independent cases on the seventh and eighth day.

### *Morphology and Development in the Spleen.*

(*a*) *Intra-corpuscular.*—No definite merozoite forms as described by Chagas were ever seen entering the red cells, and the small inclusion shown in Plate 3, fig. 3, was the only appearance observed which suggested a merozoite. Speaking generally, the smallest intra-corpuscular forms met with took the shape of very small rings with two chromatin masses, and were about one-third the size of the red blood cell. Nearly all stages from this to a fairly mature trypanosome, contained within the limiting envelope of the red cell, could be traced, the intermediate stages in many cases being similar to those of *Schizotrypanum cruzi.* This fairly mature trypanosome, however, possessed neither undulating membrane nor flagellum. Some of the more mature forms were coiled up or S-shaped, every part of them being wholly within the corpuscle. These varieties were not so numerous as the complete ring forms. The blepharoplast was not recognisable in all cases, but, when present, was usually situated at the thinnest part of the ring.

(*b*) *Extra-corpuscular.*—Ring forms were also met with free in the plasma, and these impressed one as being possibly encysted and likewise showed a development similar to that seen in the red cell. Numerous small solid, encysted (?) forms appeared in the spleen on the fourth day in one gerbil, and these, as a rule, were also present in other animals in the bone-marrow and axillary glands on and after the sixth day. The very small types appeared as spherical masses of densely-stained blue protoplasm, the nucleus in some being indicated by a small undefined violet mass, while the blepharoplast was detached.

Round the spherical body here described there existed, as a rule, at least when development was somewhat advanced, a clear area which gave the impression that the protoplasmic mass was lying in some form of vacuoloid space which was possibly surrounded by a limiting membrane. It was in connection with this membrane (?) that the blepharoplast was found, hence the use of the term "detached" (Plate 3, fig. 15). It was found that, as the protoplasmic mass became larger, a clear area developed within it. This increased in size, the nucleus of the mass became more distinct, and finally the blepharoplast appeared gradually to approach the mass until it became part of it. Such a movement on the part of the blepharoplast is well-nigh inconceivable unless there was originally some connection between it and the main mass of protoplasm. Such, however, I have never been able to demonstrate.

Eventually the spherical masses became ring-shaped, resembling in every respect those met with in the red cells.

These stages were always observed within the vacuoloid space, which enlarged to accommodate the requirements of the developing trypanosome. As in the red cells, forms almost mature were eventually found. These, which possessed free extremities, often assumed S shapes within a faintly defined capsule, the nucleus and blepharoplast being situated as in an ordinary trypanosome. Later observations showed that these forms were invariably found free in the plasma of the spleen, bone-marrow and axillary gland several days previous to the appearance of the intra-corpuscular forms.

### *Forms found in Lung Smears.*

The probability of schizogony occurring in the lung as in the case of *Schizotrypanum cruzi* was borne in mind, but no definite schizonts were seen in any of the smears. Yet the forms figured by Chagas* and described by him as parasites in the lung of vertebrates preparing for schizogony were exceptionally numerous in the lung of a gerbil on the sixth day. In fact they were the only forms present in smears from the lung at that time, but no suggestion of merozoite formation was ever observed. Some forms seen resembled the so-called latent bodies of *Trypanosoma gambiense* described by Moore and Breinl† and more recently by Fantham‡ as occurring both in *T. gambiense* and *T. rhodesiense* infections. Their method of formation, however, seems to be somewhat different to that described by these authors. It is, perhaps, sufficiently indicated by the cycle of events shown in Plate 3, figs. 19—32. In addition there was markedly evident the presence of individual spherical forms dividing into two, and occasionally three, separate bodies, each of which eventually assumes a trypanosome shape. As a result, large numbers of what were undoubtedly very young trypanosomes, each with a well-defined nucleus but without undulating membrane and flagellum, were present in the lung smears.

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* Chagas, C., "Ueber eine Neue Trypanosomiasis des Menschen," 'Memorias do Instituto Oswaldo Cruz,' August, 1908, vol. 1, No. 2.

† Moore, J. E. S., and Breinl, Anton, "The Cytology of the Trypanosomes," 'Annals Tropical Med. and Parasitol.,' July, 1907, vol. 1.

‡ Fantham, H. B., "The Life-history of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* as seen in Rats and Guinea-pigs," 'Roy. Soc. Proc.,' B, 1910, vol. 83.

No definite intra-corpuscular or free encysted forms were ever seen in the lung or peripheral blood. The former appeared exclusively in the spleen, while the latter were found without fail in the spleen, bone-marrow and axillary glands.

The axillary gland showed an unending variety of young trypanosomes corresponding in the main to those described above in the lung, while the encysted stages were also very numerous.

A marked feature, perhaps worthy of note, was the constant presence of free chromatin granules in nearly all the smears from the second day onwards. These were apparently derived from the nuclei of disintegrating trypanosomes, and possibly represented a first stage in development.

## DESCRIPTION OF PLATE 3.

Magnified 2000 diameters. Drawn with Zeiss Oc. No. 12, obj. oil imm. 2 mm., and tube length 155.

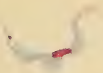
Fig. 1.—Long form with free flagellum.

Fig. 2.—Stout granular form with very short flagellum.

Figs. 3—13.—Intra-corpuscular cycle as seen in spleen on sixth day.

Figs. 14—18.—Extra-corpuscular and apparently encysted forms in spleen, sixth day. No. 14 shows granules and very small bodies, the former possibly representing infective granules, the latter possibly a very early stage of development.

Figs. 19—32.—Forms found in lung smear on sixth day. 19—25 show possible formation of so-called "latent body" of Moore and Breinl. 26—32, metamorphosis of latent body into trypanosome.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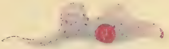
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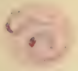
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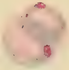
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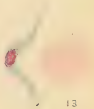
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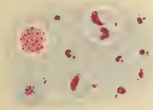
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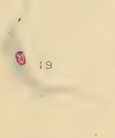
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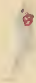
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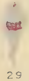
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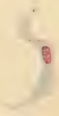
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Grout, sc. & imp

# *A Contribution to our Knowledge of the Protozoa of the Soil.*

By T. Goodey, M.Sc. (Birm.), Mackinnon Student of the Royal
Society, Rothamsted Experimental Station.

(Communicated by A. D. Hall, F.R.S. Received May 19,—Read June 1, 1911.)

[Plate 4.]

CONTENTS.

PAGE
Introduction .......... 165
Part I.—Protozoa Found.
  *a.* Methods .......... 167
  *b.* Systematic .......... 168
  *c.* Active Protozoa Found on the Surface of the Soil .......... 169
Part II.—Experimental.
  *d.* Thermotaxis .......... 170
  *e.* Galvanotaxis .......... 171
  *f.* Excystation Experiments .......... 177
  *g.* Summary and General Conclusions .......... 178
Literature and Explanation of Plate.

## *Introduction.*

In the 'Journal of Agricultural Science,' Vol. III, Part 2, 1909, Drs. Russell and Hutchinson of this laboratory published an account of their investigations on the effect of partial sterilisation of soil on the production of plant food. In this paper it is shown that, when soils are heated or treated with certain volatile antiseptics, and brought again under conditions favourable to plant growth, they show a great increase in fertility. It is further shown that, although the bacteria are at first reduced very considerably in numbers, yet under conditions of temperature, moisture, and aëration favouring growth, they subsequently increase enormously in numbers. *Pari passu* with this increase in the number of bacteria, there is an increase in the production of ammonia in the soil, and it is to this that the soil owes its greater power of production.

In explanation of these results, the theory is advanced that the treatment by heating or with volatile antiseptics has removed some factor which in the untreated soil normally limits the growth of bacteria, and thus the rate of ammonia production. This limiting factor is looked upon as being biological in character, but not of a bacterial nature.

It was found in cultures of untreated soil in suitable nutrient media that certain animal organisms belonging to the phylum protozoa became very abundant a day or two after the culture had been made. On the other hand, cultures of treated soil failed to reveal these protozoa, which are much more susceptible than the bacteria to the sterilising agents employed.

These organisms included certain amœbæ, flagellates, and infusorian ciliates, known to be devourers of bacteria *when living in a liquid medium.*

To these protozoa, therefore, the authors assign the function of limiting the activity of bacteria in the soil, though they point out* that they by no means wish to imply that they are the only limiting factor.

Various workers have recorded the presence of protozoa in the soil, especially of amœbæ. Greeff† gives an account of four species of amœba which he found in association with damp earth and moss, etc. Beijerinck,‡ Celli,§ Frosch,|| Tsujitani¶ describe certain small amœbæ which they obtained in cultures made from garden soil, and give methods for obtaining these organisms in pure culture on various nutrient media of a solid nature. Störmer** found that several colonies of amœbæ developed in plates inoculated with 1 mgrm. of soil.

Hiltner†† speaks of finding numerous amœbæ, flagellates, and ciliated protozoa in cultures made from soil, and suggests that the presence of these animal organisms has some vital connection with the properties of the soil.

Hartmann and Nägler,‡‡ and Nägler,§§ have published important contributions to our knowledge of the life-history and nuclear changes of several small amœbæ, three or four of which came from cultures made with soil.

Wolff |||| speaks of a characteristic protozoan fauna in certain irrigated soils. He attributes various functions to the protozoa, viz.:—(1) Carrying disease germs; (2) taking up and killing algæ, fungi, and bacteria; (3) absorption of useful material from the soil water, thus preventing it from sinking to the deeper layers of soil; and (4) power of living at all seasons of the year, so long as the ground is sufficiently moist and is not frozen. He gives a plate with drawings of protozoa.

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* *Loc. cit.*, p. 142, § 42.

† Greeff, R., 'Arch. für Mikroskop. Anat.,' 1866, vol. 2, p. 299.

‡ Beijerinck, 'Centr. für Bakt.,' 1896, vol. 19, Pt. 1, p. 257.

§ Celli, *ibid.*, p. 1025.

|| Frosch, P., 'Cent. für Bakt.,' 1897, vol. 21, Pt. 1, p. 579.

¶ Tsujitani, J., 'Cent. für Bakt.,' 1898, vol. 24, Pt. 1, p. 666.

** Störmer, K., 'Jahrsber. d. Vereinigung f. angewandte Botanik,' 1907, No. 5, p. 113.

†† Hiltner, L., *ibid.*, p. 200.

‡‡ Hartmann, M., Nägler, K., 'Sitz. Ber. d. Naturf. Freunde,' 1908, Berlin, vol. 4.

§§ Nägler, K., 'Arch. für Protistenk.,' 1909, vol. 15, p. 1.

|||| Wolff, 'Mitt. a. d. Kaiser Wilhelm-Instit. f. Landw. Bromberg,' 1909, vol. 1, Heft 4, p. 382; Abst., 'Cent. f. Bakt.,' 1909, vol. 24, p. 465. (Only abstract seen.)

Peck* found protozoa in the mannite medium used for the cultivation of Azotobacter from soil.

However, no comprehensive account of the various species of protozoa found in the soil has as yet been published, and, in view of the fact that such highly important functions have been attributed to them, it is very desirable that our knowledge of them should be extended. It is with this object in view that the present paper is put forward as a preliminary communication.

## Part I.—*Protozoa Found.*

(a) *Methods.*—The method of cultivation which has been chiefly used is as follows:—To a quantity of sterile 1-per-cent. hay infusion† contained in a small Erlenmayer flask or test tube are added a few grammes of the soil, the protozoa of which it is desired to investigate. The mixture is shaken up, a plug of cotton wool inserted in the mouth of the vessel, and the culture placed in the incubator either at 20° or 30° C. After allowing it to stand for a day or two, numerous protozoa can be taken from the surface of the liquid.

As an *intra-vitam* stain, neutral red diluted 1/100,000 has been found very useful for showing up food vacuoles. For instantaneously killing the organisms with their flagella or cilia fully displayed, the preparation is exposed to the action of osmic vapour for a few seconds. A saturated solution of methyl green in 1-per-cent. acetic acid has proved very serviceable for quickly staining and showing up nuclei. It has been used in the following manner:—A small drop of the culture solution is taken on the sterilised platinum loop, and spread out on a cover-slip. This is then exposed to the action of osmic vapour for a short time and a small drop of methyl-green solution is added. The cover-slip is then inverted over a cavity slide or placed on an ordinary slide and examined. It can be waxed down by painting round its edge with the hot wick of a candle, the flame of which has been just previously blown out, and thus kept from drying up for a long time.

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* Peck, S. S., 'Bull. 34, Hawaiian Sugar Planters' Expt. Stat.,' September, 1910.

† The hay infusion used throughout has been slightly alkaline in reaction; sufficient N/1 NaOH solution being added to the boiled and filtered liquid to render it just alkaline to litmus. Other media found useful, especially for film preparations, are 1-per-cent. hay infusion + egg albumen, 0·75 per cent. NaCl solution + egg albumen; to each 100 c.c. of liquid is added in each case about 15 c.c. of fresh white of egg. Cultures of amœba and other protozoa can be made on the agar medium made up according to the recipe given by Berliner ('Arch. für Protistenk.,' 1909, vol. 15).

A filtered soil extract has also been used as a liquid medium.

(b) *Systematic.*—The following list of organisms includes all that it has been possible to classify and name up to date. The scheme adopted is simple, all the different families, sub-families, etc., to which any particular organism belongs are not given.

It must not be supposed that all the forms have been found in any one culture; some cultures may yield a large number of different species, whilst others give only a few. Some species, *e.g.*, *Cercomonas* and *Colpoda cucullus* and *Col. steinii*, are very commonly met with, whilst others only occasionally occur. Notes have been added to the different species tabulated, indicating their abundance, etc.

Class.—Mastigophora (Dies).
Order.—Protomonadina (Blochmann).

1. *Cercomonas sp.?* (Duj. em. Bütschli). (Pl. 4, fig. 1.)
2. *Dimorpha radiata* (Klebs).

These forms have two flagella arising from the nuclear area; they ingest food by amœboid movement and are very common, occurring in practically all cultures and from all soils.

3. *Oicomonas* (Kent), two species, one much larger than the other, occasionally found.
4. *Bodo lens* (Ehrb.), has occurred in a few cultures, from a pasture soil.

Order.—Euglenoidena.

5. *Anisonema sulcatum* (Duj.), found in culture from one particular soil.

Family.—Choanoflagellidæ (Stein).

6. *Codosiga botrytis* (Ehrb.), only seen on one occasion, in a culture from a pasture soil.

Two other flagellates have been encountered, but so far it has not been possible to classify them.

Class.—Rhizopoda (von Siebold).
Order.—Lobosa.

7. *Amœba* "*limax*" (Pl. 4, fig. 2).

The amœbæ found are all placed provisionally in the "*limax*" group on account of their size, and lobose pseudopodia. They are of frequent occurrence and vary considerably in size; some are 8—10 $\mu$ in diameter, others 20—40 $\mu$, whilst one form reached 100 $\mu$ in length. Before these can be definitely named according to their particular species, it will be necessary to work out the structure of the nucleus and to follow the nuclear changes in division, etc. The recent work of Nägler (*loc. cit.*) and others on different amœbæ of the "limax" group shows this to be essential.

Order.—Monothalamia (M. Schultze).

8. *Arcella vulgaris* (Ehrb.), in an old culture of sewage soil.
9. *Difflugia globulosa* (Duj.), shell built up of cells of dead algæ, in an old culture of sewage soil.
10. *Trinema enchelys* (Ehrb.), in an old culture of a manured arable soil.
11. Order.—*Mycetozoa;* numerous active zoospores have been encountered in a few cultures, from arable, pasture, and garden soils.

Class.—Ciliata.
Order.—Holotricha (Stein.).

12. *Spathidium spathula*, only occasionally found, from manured arable soil.

13. *Enchelys sp. ?*, a small form, occasionally found from arable and pasture soil.
14. *Chilodon sp. ?* (Ehrb.) (Pl. 4, fig. 3), fairly common from arable and pasture soil. Has only two contractile vacuoles.
15. *Colpoda cucullus* (O. F. M.) (Pl. 4, fig. 4), very widely distributed and extremely common in arable and pasture soil.
16. *Colpoda steinii* (Maup. em. Enriques) (Pl. 4, fig. 6), as common as *Col. cucullus.*
17. *Colpoda maupasii* (Enriques), only found once or twice from a pasture soil.
18. *Balantiophorus elongatus* (Schew.) (Pl. 4, fig. 7), fairly common in arable and pasture soils.
19. *Balantiophorus minutus* (Schew.), found in culture from only one soil.
20. *Cryptochilum nigricans* (Maup.), fairly common from a manured arable soil.
21. An unclassified ciliate.

Order.—Hypotricha (Stein).

Some difficulty has been experienced in accurately placing the different species of this order. Nos. 22, 26, and 27 are only provisionally placed.

22. *Gastrostyla steinii* (Engel.), a very large form, found on two occasions from arable soil, devouring *Col. cucullus.*
23. *Urostyla grandis* (Ehrb.), found in cultures from a manured arable soil.
24. *Gonostomum affine* (Stein) (Pl. 4, fig. 9), rather widely distributed, found in many arable and pasture soils.
25. *Pleurotricha grandis ?* (Stein) (Pl. 4, fig. 8), same remarks apply as to 21.
26. *Pleurotricha lanceolata* (*?*) (Ehrb.), a long, flexible form, with pointed posterior end, from an arable soil.
27. *Oxytricha sp.* (*?*), a form from a manured garden soil.

Order.—Peritricha (Stein).

28. *Vorticella microstoma* (Ehrb.) (Pl. 4, figs. 10, 11), fairly common in occurrence, from arable soil.
29. *Vorticella putrinum* (Kent), found in cultures from a sewage farm soil.
30. *Epistylis coarctata* (C. and L.), rather large organism from a pasture soil.

(c) *Active Protozoa found on the Surface of the Soil.*—About the middle of December, 1910, some wet, rotting mangold leaves were collected from the surface of Barnfield, Plot 2. There had been an abundance of rain and the ground was very wet. Some of the water from the surface of the leaves was sucked up and then examined under the microscope. There were numerous free living *Cercomonas*, some small *Amœba limax*, a few *Colpoda cucullus* and many *Urostyla grandis;* all of them belonging to species found in soil cultures.

On other occasions, wet grass leaves and stalks from the surface of the soil, or from very close to it, have been examined. On these I have found active *Cercomonas, Colpoda cucullus, Amœba verrucosa, Amœba limax, Epistylis coarctata,* and a small hypotrichous ciliate which I have not been able to classify.

One would, of course, expect to find active protozoa in situations such as these, where there is an abundance of moisture for them to swim about in.

Activity on the surface of the soil must not, however, be confused with activity in it.

## Part II.—*Experimental.*

Perhaps the most important point to determine is the state in which the protozoa are present in the soil. We know they are there, but to exercise the function which has been attributed to them by Russell and Hutchinson they must be present in a free-living, active condition, capable of a certain amount of movement consequent upon their need for food, moisture and oxygen.

On account of the opacity and texture of the soil, it presents insuperable difficulties in the way of direct microscopic observation of such minute organisms as protozoa. Even when finely teased out in a liquid and spread in a thin layer in a glass trough, it has never been possible to find free-living organisms.

Further, the method employed for cultivating them described above does not throw any light upon their condition. There is nothing to tell us whether the forms which occur in the culture have developed from a free-living or from an encysted condition.

For the elucidation of this question various experiments have been carried out, both "indirect," *i.e.* designed to give certain data on which inferences could be based as to the condition of the protozoa, and "direct," *i.e.* designed to affect the activities of any free-living protozoa in the soil and induce them to leave it.

In the following pages an account is given, first, of the direct attempts, and second, of an indirect method which has proved very useful.

(d) *Thermotaxis.*—A piece of apparatus was constructed on the same principles as that described by Jennings.* It consisted of three glass tubes about 8 mm. in diameter, supported in a horizontal position at exactly the same level, by being passed through holes in a block of wood. The tubes were placed 1 inch apart and so arranged that a glass slide rested evenly on each, giving complete contact of the surfaces. Indiarubber tubes were connected to the ends of the glass tubes and attached, on the inflow side, to water taps, one supplying water at about 15° C. and the other leading from a geyser, by means of which water at any required temperature from 15 to 60° C. could be obtained. The waste water was led off by rubber tubes emptying into a sink.

A glass trough was made by cementing strips of glass to a slide 3 inches by 1·5 inch with Canada balsam; the trough was about 6·5 cm. long, 1·5 cm.

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* H. S. Jennings, 'The Behavior of Lower Organisms,' Carnegie Institution, Washington, 1904, No. 16, p. 11.

wide, and 2 mm. deep. Examination of the contents of the trough was carried out with a powerful hand lens and with low power objectives. The preliminary trials with this apparatus, using active free-swimming Colpoda and Pleurotricha in a culture, proved that the method was not precise enough.

A second piece of apparatus was then devised, similar in essentials to the first, but, in place of the glass tubes, three zinc tubes $\frac{3}{4}$ inch square in section were substituted. These were corked at each end and through the corks glass tubes were passed, and to these, rubber tubes gave connection with the water supply, etc., as before. The zinc tubes were clamped in a horizontal position between two strips of wood, and carefully arranged so as to give good contact with the glass trough when placed on them. *Colpoda cucullus* and *Pleurotricha grandis* in a culture liquid were found to respond quite well, especially when the trough was slowly moved more and more on to the tube conveying water at the higher temperature. The tropism was always negative, the organisms moving away from the source of heat towards the region of lower temperature. The movement was fairly precise and general, but there were always a few stragglers, apparently swimming about quite indefinitely.

A culture containing numerous active ciliated protozoa was mixed with a small quantity of sterilised soil, the mixture put in the glass trough and placed on the tubes. The object of this was to see if it were possible to cause active ciliates to leave the soil particles and collect in the clear liquid at one end of the trough. No satisfactory results were obtained, the presence of soil particles in the liquid appearing to prevent the organisms from responding to the stimulus of heat. They could shelter behind and under the tiny masses of soil, and there can be little doubt that local disturbances of temperature were set up also. Moreover, it must be borne in mind that the object of the experiments was to attempt to discover any free-living protozoa in fresh soil. For this reason it was not possible to carry on the experiment for any length of time, owing to the fact that encysted forms might hatch out and so mar the results. Fresh soil was taken on one or two occasions and teased out in sterile 1-per-cent. hay infusion in the glass trough, and treated as described above, but no free-living protozoa were found. The method was therefore discontinued.

(e) *Galvanotaxis.*—Verworn* gives an account of the way in which certain protozoa respond to the stimulus of a continuous electric current. It is there shown that when a liquid containing amœbæ or certain ciliated protozoa has a continuous current passed through it by means of two non-polarisable electrodes, the organisms move through the liquid and assemble at the cathode. On these lines it seemed possible to devise a method of applying the electric stimulus to the soil protozoa, and cause them to leave the soil and come to a point convenient for observation, assuming, of course, that they were present in a free-living condition.

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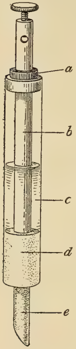
* 'General Physiology,' English translation, 1899, p. 455.

In preliminary experiments, the current was passed into the culture liquid through two non-polarisable electrodes (see fig.) similar to those described by Verworn.* Each consisted of a glass tube about 8 cm. long by 6 mm. in diameter, one end of which was plugged with fresh moist clay,† and into this a tip of porous clay was inserted.

A concentrated solution of zinc sulphate was placed inside the tube and into this an amalgamated zinc rod dipped, kept in position by means of a rubber band surrounding it and fitting the mouth of the tube. The upper end of each rod was fitted with a screw so as to serve as a terminal. By inserting a milliammeter in the circuit the current passing could be read off.



Non-polarisable electrode.

*a.* Rubber band.
*b.* Amalgamated zinc rod.
*c.* Concentrated zinc-sulphate solution.
*d.* Moist clay plug.
*e.* Tip of porous clay.

Actively swimming ciliated protozoa in culture liquid quickly responded to the current and collected at the cathode. The presence of soil-particles, however, in any considerable quantity prevented the ciliates from assembling rapidly, and for this reason it did not seem possible to cause protozoa to collect at a given point by directly applying the current to the soil.

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* *Loc. cit.*, p. 455.

† Care must be taken to renew the moist clay plugs each day during their use, and to keep the porous clay tips clean and free from $ZnSO_4$ which gradually soaks into them.

The following method was finally arrived at:—In a large Petri dish, 11 cm. in diameter and 2 cm. deep, a fairly large quantity (2—5 grm.) of the required soil is placed: 20 c.c. of sterile 1-per-cent. hay infusion is then added and the soil quickly teased out as finely as possible. The culture is then put into the 30° C. incubator—incubation has in all cases been carried out at this temperature. After a time, the culture is carefully taken from the incubator and the supernatant liquid pipetted off, without much agitation, so as to avoid taking up any quantity of soil particles.

This liquid is put into the two tubes of a small hand centrifugal machine, and centrifugalised at high speed for a few minutes in order to bring down any protozoa present to the bottom of the tubes. The bulk of the liquid is then taken up as quickly as possible by means of a pipette, and put back into the culture in the large dish. The remaining few cubic centimetres at the bottom of the tubes are then agitated and taken up by a small pipette and put into a shallow Petri dish 6 cm. in diameter. This is placed on the stage of the microscope, and submitted to the electric current for a few minutes. The current passes between the two non-polarisable electrodes dipping into the liquid and connected up with the storage battery and the milliammeter. An E.M.F. of 12 volts and a current of 0·0002 to 0·00028 ampère have been used in most of the work. By focussing a low-power objective (1-inch) on to the cathode or close to it, it is possible to watch the ciliates come swimming into this region under the influence of the electric stimulus.

It has been possible by this means to find, in a few minutes, a few or even solitary ciliates in 5 or 6 c.c. of culture liquid. If, after the passage of the current for about 5 minutes, no protozoa are found in the region of the cathode, the conclusion is that none are present, and the liquid is put back into the main culture.

For further observation, under high-power objectives, of the ciliates caused to aggregate at the cathode, it has been necessary to extract them from the culture liquid. For this purpose capillary pipettes have been used. Placing the fine end of one of these under the low power, and then having the required organism in focus, following it carefully by moving the dish with the left hand, the pipette is brought down on to it, with the result that the organism is suddenly sucked up into the tube. From this it is usually transferred to a cover-slip by blowing down the broad end of the tube. Here it can be treated in whatever way is desired.

As mentioned in Part I, the protozoa in the ordinary soil culture do not occur in any abundance until two or three days after inoculation. It was necessary, therefore, to determine, if possible, the period of time elapsing between inoculation and the appearance of the first active protozoa in the culture.

Preliminary experiments revealed the fact that active ciliated protozoa could be obtained in a soil culture in the short time of four and a half hours, and by using the galvanotactic method just described it has been possible to obtain a considerable amount of information.

In the following experiments, different soils have been used and comparatively large quantities have been taken in order to increase the chance of finding any possible free-living protozoa in the soil. The larger the quantity of soil, within limits, the more probable is it that active protozoa, if present, will come out into the culture liquid.

No. 1.—Manured soil from Barnfield, very moist.

After 1 hr. 40 m.—1 *Colpoda cucullus*, only seen once, protoplasm rather granular at posterior end.
1 *Vorticella microstoma*, free swimming, cylindrical, aboral ciliary ring, no food vacuoles, protoplasm clear.
„ 4 „ 0 „ —1 *Enchelys sp. ?*, protoplasm clear.

This soil had been stored for six weeks under conditions favourable to bacterial activity, and presumably to protozoal activity also.

No. 2.—Same soil as in 1.

After 4 hr. 25 m.—1 *Col. cucullus*, protoplasm rather granular, not stained with neutral red, no food vacuoles.
Many *Col. steinii*, protoplasm clear.
3 or 4 *Gonostomum affine*, protoplasm very clear, nuclear areas easily visible.

No. 3.—Manured soil from Barnfield.

After 0 hr. 40 m.—No active forms.
„ 1 „ 30 „ —1 *Col. cucullus*, protoplasm rather granular, no food vacuoles.
1 *Col. steinii*, rather granular, nucleus very clear on staining.
1 *Balantiophorus elongatus*, protoplasm clear.
„ 3 „ 0 „ —1 *Col. cucullus*, 2 or 3 *Col. steinii*, 1 *Gonostomum affine*, 2 *Balantiophorus elongatus*, 2 *Spathidium spathula*.

This soil had been air-dried, subsequently moistened and stored at laboratory temperature since October, 1910, in a bottle plugged with cotton wool.

No. 4.—Manured soil, Hoosfield, fresh and moist.

After 1 hr. 20 m.—No active forms.
„ 2 „ 0 „ — „ „
„ 3 „ 45 „ —1 *Col. steinii*, very active, protoplasm clear.
1 *Bal. elongatus*, protoplasm clear.

No. 5.—Manured soil as in 1.

After 0 hr. 30 m.—No active forms.
„ 1 „ 15 „ — „ „
„ 2 „ 15 „ — „ „

No. 6.—Manured greenhouse soil from Waltham Cross, moist.

After 1 hr. 10 m.—No active forms.

„ 6 „ 10 „ —1 or 2 *Col. cucullus*, many *Col. steinii* and *Gonostomum affine*, all having protoplasm clear and finely granular in appearance.

This was a "sick" soil, *i.e.*, it no longer possessed the power of yielding heavy crops. The factor limiting bacterial activity was exerting itself fully.

No. 7.—Soil as 6.

After 1 hr. 35 m.—No active forms.

„ 3 „ 20 „ — „ „

„ 7 „ 10 „ —Many active *Col. steinii* and *Gonostomum affine.*

No. 8.—Soil as in 6 and 7.

After 1 hr. 30 m.—No active forms.

„ 4 „ 0 „ —Several *Gonostomum affine*, 1 or 2 *Col. steinii*, and 1 *Vorticella microstoma*, free swimming, cylindrical, aboral ciliary ring. Protoplasm of all organisms clear, no food vacuoles.

No. 9.—Manured soil from Barnfield, fairly moist.

After 1 hr. 40 m.—1 or 2 *Col. steinii*, 1 *Bal. elongatus*, protoplasm clear.

„ 2 „ 30 „ —5 or 6 *Col. steinii*, 2 or 3 *Bal. elongatus.*

„ 5 „ 20 „ —Many *Col. cucullus*, *Col. steinii*, *Gonostomum affine*, 1 or 2 *Bal. elongatus* and *Enchelys.*

The soil had been stored in the same way as 3 since October, 1910.

No. 10.—Manured soil from Barnfield, fresh and moist.

After 0 hr. 25 m.—No active forms.

„ 1 „ 5 „ — „ „

„ 2 „ 0 „ — „ „

„ 4 „ 35 „ —1 *Col. cucullus*, 2 *Bal. elongatus*, protoplasm clear and finely granular.

No. 11.—Soil same as 9.

After 0 hr. 50 m.—No active forms.

„ 1 „ 30 „ —1 *Col. cucullus*, protoplasm clear, nucleus easily seen, without staining, under high power.

„ 3 „ 20 „ —2 *Col. cucullus*, protoplasm clear, one caught showing nucleus well, without staining.

„ 4 „ 30 „ —1 *Col. cucullus*, dark in appearance, having a fair number of food vacuoles.

4 or 5 *Gonostomum affine*, 2 caught showing clear protoplasm and nuclei without staining.

No. 12.—Soil same as 10.

After 1 hr. 20 m.—No active forms.

„ 2 „ 15 „ —2 *Col. steinii*, protoplasm clear.

„ 5 „ 0 „ —1 *Col. steinii*, 1 or 2 *Bal. elongatus*, protoplasm clear, 1 *Gon. affine*, nuclei showing well without staining.

„ 7 „ 0 „ —Many *Col. cucullus*, *Col. steinii*, *Bal. elongatus*, all having clear protoplasm, and a few food vacuoles.

1 *Epistylis coarctata*, cylindrical, free swimming, 1 or 2 food vacuoles.

No. 13.—Soil, a mixture of toluene-evaporated + 50 per cent. untreated soil, fairly moist.

After 1 hr. 10 m.—No active forms.

„ 1 „ 55 „ —1 *Bal. elongatus.*

„ 3 „ 30 „ —No active forms.

„ 6 „ 0 „ —1 *Col. cucullus*, 5 or 6 *Col. steinii*, protoplasm clear and nuclei showing well.

The soil used in the following tabulated Experiments 14–18 had received the following treatment: It was first *partially sterilised* with toluene, and the antiseptic thoroughly evaporated. This treatment brought the water content of the soil down to 3 or 4 per cent. By adding distilled water, the water content was brought up to 10 per cent., and the soil was left in a corked bottle for two days, in order to allow the water to distribute itself evenly The water content was next brought up to approximately 20 per cent., by the addition of a culture of hay infusion which contained an abundance of active *Colpoda cucullus* and *Pleurotricha grandis.* The soil was spread out in a thin layer, and the liquid added as evenly as possible by means of a fine pipette. After this it was put up in a small bottle plugged with cotton wool, and kept either at 20° C. or at laboratory temperature. The purpose of this treatment was to give every opportunity for the active ciliates added to continue in their free-swimming condition, if that were at all possible. For this reason the culture was not added directly to the dry soil, as it was thought that the bulk of the liquid, on being taken up rapidly by the soil, would do considerable violence to the rather delicate protoplasm of the protozoa, hence the moistening up to 10-per-cent. water content was carried out before the addition of the culture.

No. 14.

After 1 hr. 30 m.—No active forms.
„ 2 „ 40 „ —1 *Col. cucullus*, protoplasm fairly clear, a few granules, no food vacuoles.
„ 4 „ 5 „ —No active forms.

No. 15.

After 1 hr. 30 m.—No active forms.
„ 3 „ 45 „ —1 *Col. cucullus*, protoplasm rather granular.
„ 6 „ 15 „ —1 *Col. cucullus*, 1 *Col. steinii*, protoplasm fairly clear.
„ 7 „ 20 „ —No active forms.

No. 16.—The above soil, air-dried.

After 3 hr. 20 m.—2 *Col. cucullus*, protoplasm clear, one with 3 or 4 food vacuoles.
„ 4 „ 10 „ —No active forms.
„ 5 „ 15 „ —2 *Col. cucullus*, not captured.
„ 16 „ 0 „ —Many *Col. cucullus.*

No. 17.—8·6 grm. of the above soil, air-dried.

After 4 hr. 0 m.—1 *Col. cucullus*, protoplasm clear, no food vacuoles, very distinct nucleus without staining.
„ 6 „ 15 „ —3 *Col. cucullus*, two rather small, protoplasm fairly clear, with a few food vacuoles; one larger and with quite a large number of food vacuoles.
„ 8 „ 0 „ —2 *Col. cucullus.*

No. 18.—8·6 grm. of the soil, undried.

After 1 hr. 45 m.—No active forms.
„ 4 „ 35 „ —2 *Col. cucullus.*
„ 7 „ 15 „ —1 *Pleurotricha grandis*, fairly numerous food vacuoles.

(f) *Excystation.**—Concurrently with the above experiments with soil, a series on the development of free-swimming ciliated protozoa from their resting cysts have been carried out. These have been done chiefly with the cysts of *Colpoda cucullus.*

Rhumbler† speaks of obtaining active *Col. cucullus* by excystation from "dauercysten" in about six hours. It became necessary, therefore, to determine the length of time required for the excystation of *Col. cucullus.*

A supply of resting cysts was obtained from the sides of a flask containing an old culture of this ciliate, and a large number of these cysts were slowly air dried on a filter paper. Undried cysts have also been used. The experiments have been conducted chiefly in hanging drop cultures, either over cavity slides or glass rings, and incubation has in all cases been carried out at 30° C.

During excystation the outer wall of the cyst, ectocyst, which is generally rather rough and is very resistant, ruptures and permits the transparent-walled endocyst to come out. Within the latter the organism begins to rotate, and it is seen that the contractile vacuole begins to pulsate. The endocyst gradually swells up and its wall gets thinner and thinner (Plate 4, fig. 5). During this process, the cilia of the Colpoda are moving rapidly, causing it to revolve within the endocyst, and setting in constant motion the mass of defæcated excretory matter extruded during encystation. Finally the endocyst wall gives way and the organism swims away freely.

The protoplasm is generally finely granular in appearance, and in the endoplasm there are varying quantities of larger granules. Sometimes the latter are quite large and rather dark in appearance.

It is almost always possible for one to distinguish quite easily the situation of the meganucleus as a spherical area, more dense in appearance than the rest of the protoplasm.

There are, of course, no food vacuoles in the endoplasm, for these are only found after the organism has been obtaining food for some time.

No. 1.—*Col. cucullus* emerged in 4 hr. from dried cysts.
" 2.—*Col. cucullus* emerged in 4½ hr. from dried cysts and in 6¼ hr. from undried cysts.
" 3.—Many *Col. cucullus* emerged in 3 hr. 25 m. from dried cysts.
" 4.—One or two *Col. cucullus* emerged in 3½ hr. from undried cysts.
" 5.—One *Col. cucullus* emerged in 2 hr. 35 m. from dried cysts which had been standing on a filter paper moistened slightly with hay infusion on the previous evening.
" 6.—One *Col. cucullus* emerged in 2 hr. from cysts treated as in 5, only moistened for about 30 hr. with hay infusion.

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* This word is used to designate the stages up to and including the emergence of active protozoa from their resting cysts.

† Rhumbler, L., 'Zeitschr. für wissen. Zool.,' 1888, vol. 46, p. 571.

The foregoing experiments with soils and resting cysts show that, as regards *time*, the first ciliated protozoa were revealed in soil cultures only after about one and a half hours. This is the shortest time observed, and no ciliated protozoa have been found earlier than this; as a rule the incubation period is rather longer. The incubation period for the excystation of *Col. cucullus* from resting cysts is from two to four hours.

*In appearance*, the first ciliated protozoa to occur in soil cultures are clear, and have a finely granular protoplasm with a varying quantity of larger granules. They have no food vacuoles, and it is often possible, without staining, to distinguish the nuclear area as a denser region of protoplasm. In one or two cases the meganucleus with its micronucleus has been well seen in *Col. cucullus* without staining. Especially noteworthy are the three free-swimming, cylindrical vorticellid forms (Plate 4, fig. 11). The ordinary form of Vorticella is stalked and attached, whilst the cylindrical, free-swimming form, without food vacuoles, is characteristic of but recent emergence from a resting cyst.

Recently excysted *Col. cucullus* are easily recognisable by their clear protoplasm, their readily distinguishable nuclear area, and by the absence of food vacuoles.

(g) *Summary and Conclusions.*—1. Given the opportunity for ciliated protozoa to live actively and multiply on the surface of the soil, with the production of resting cysts on the occurrence of conditions adverse to further growth, it is possible to account for the ciliates which develop in soil cultures. The resting cysts produced on the surface would gradually be washed down into the soil, where they would remain. Many of these would, no doubt, lose their vitality after a long time, whilst others would be capable of excystation when once they came to the surface and conditions favourable to their growth prevailed.

2. The incubation periods observed for the first ciliated protozoa which appear in soil cultures (1½ to 3 or 4 hours) and for the earliest Colpoda to emerge from resting cysts (2 to 4 hours) are comparable. Comparable periods required for appearance indicate a similarity in the condition of the protozoa at the commencement of the experiment, viz., the encysted condition.

3. The first Colpoda to occur in soil cultures are very similar in appearance to those which have just emerged from resting cysts. The protoplasm is generally clear, the nuclear area is distinct and easily visible without staining, and no food vacuoles are present. If the Colpoda had been free-living and actively devouring bacteria in the soil, they must certainly have possessed food vacuoles. This indicates that the ciliated protozoa of the soil are present only in the condition of resting cysts.

4. Soils stored under conditions very favourable to bacterial activity, and presumably to protozoal activity, if such is possible in the soil, give every indication that the first ciliated protozoa to occur in cultures have developed from resting cysts. Moist soil fresh from the field only yields ciliates slowly.

The first Colpoda to appear from the soil to which they had been added in active condition, only occur after sufficient time has elapsed for emergence from resting cysts. The protoplasm resembles that of recently excysted Colpoda. These facts indicate that the free-living organisms added to the soil had not remained in the active condition, but had encysted.

*General Conclusion.*—The ciliated protozoa which are so characteristic a feature of cultures made from soil only exist in the soil in an encysted condition. In consequence, they cannot function as the factor limiting bacterial activity in the soil.

From the nature of the methods employed in the galvanotaxis experiments it is only possible, as yet, to deal with ciliated protozoa; amœbæ and flagellates are not dealt with. For this reason the inferences drawn only apply to ciliates.

In conclusion, I desire to offer my best thanks to Mr. A. D. Hall, and to the Lawes Agricultural Trust, for the facilities afforded me to carry out the work at this station. Especially grateful am I to Dr. H. B. Hutchinson, with whom I have been intimately associated during the course of the work, for the unfailing assistance which he has given me, both by suggestion and criticism. To Prof. F. W. Gamble I should also like to express my thanks for bringing me into touch with the work and for much valuable criticism. I am also indebted to the Birmingham Natural History and Philosophical Society for a grant from the "Endowment of Research Fund."

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## EXPLANATION OF PLATE 4.

The figures were all drawn with the aid of the camera lucida.

Fig. 1.—*Cercomonas sp.?* ×1200. Stained with methyl green, showing the two flagella arising from the outer nuclear zone.

Fig. 2.—*Amœba "limax."* ×3000. Stained Heidenhain's hæmatoxylin.

Fig. 3.—*Chilodon sp.* ×1200. Dorsal view, showing mega- and micro-nucleus.

Fig. 4.—*Colpoda cucullus.* ×1000. From a living example. The round bodies are food vacuoles; one is in process of formation at the mouth.

Fig. 5.—*Colpoda cucullus.* ×610. An example developing within the endocyst. Nuclear area shown as a dense circular area. Mass of excretion matter also present.

Fig. 6.—*Colpoda steinii.* ×1200. Stained with methyl green, showing mega- and micro-nucleus.

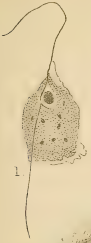
Fig. 7.—*Balantiophorus elongatus.* ×1200. Lateral view, stained methyl green, mega- and micro-nucleus showing.

Fig. 8.—*Pleurotricha grandis?* ×610. Ventral view.

Fig. 9.—*Gonostomum affine.* ×760. Ventral view.

Fig. 10.—*Vorticella microstoma.* ×610. From a living example of the stalked form.

Fig. 11.—*Vorticella microstoma.* ×760. The free-swimming, recently excysted form with aboral ciliary ring.



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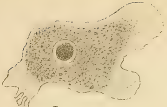

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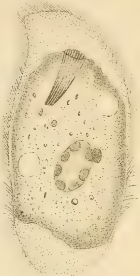

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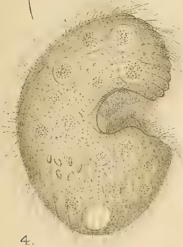

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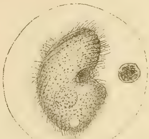

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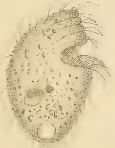

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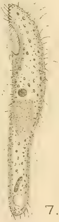

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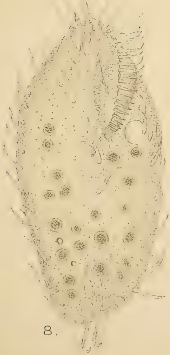

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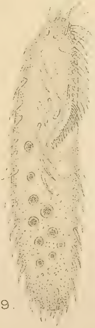

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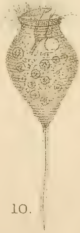

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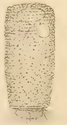

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# *The Morphology of* Trypanosoma evansi (Steel).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service.

(Received May 19,—Read June 1, 1911.)

[PLATE 5.]

## INTRODUCTION.

In previous papers published in the 'Proceedings,' the morphology of various trypanosomes, such as *Trypanosoma pecorum, vivax, uniforme, nanum,* and *brucei,* has been described somewhat more fully than is usually done. It is proposed to do the same for *Trypanosoma evansi* in this paper.

This trypanosome causes the disease in elephants, camels, horses, cattle, and dogs, known in India as Surra. It was discovered in 1880, by Evans, in the Punjab.

### A. *Living, Unstained.*

*Trypanosoma evansi* is described by Laveran and Mesnil as being more motile than *Trypanosoma brucei,* and as sometimes travelling out of the field of the microscope, which is rarely the case with *Trypanosoma brucei.*

### B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as previously described in the 'Proceedings.'*

*Length.*—The following table gives the length of this species as found in the elephant, camel, horse, dog, guinea-pig, and rat.

It will be seen from the following table that *Trypanosoma evansi* varies in length between 18 and 34 microns, the average of 820 individuals being 24·9. It is true that short, stumpy individuals, without free flagellum, are sometimes found, but very few of these rare specimens were met with in the 820 trypanosomes taken as they came for the purposes of the table. These short and stumpy forms are so few and far between that this species may be described as monomorphic, whereas *Trypanosoma brucei,* which has about 40 per cent. of these short, stumpy forms, may be called dimorphic.

*Breadth.*—This lies usually between 1·5 and 2 microns.

*Shape.*—*Trypanosoma evansi* resembles very closely the intermediate forms of *Trypanosoma brucei.* The posterior extremity is often triangular in shape, the body gradually narrowing towards the anterior extremity (Plate 5).

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of *Trypanosoma evansi.*

| No. of expt. | Animal. | Method of fixing. | Method of staining. | In microns. | | |
|---|---|---|---|---|---|---|
| | | | | Average length. | Maximum length. | Minimum length. |
| — | Elephant | — | — | 27·0 | 32·0 | 20·0 |
| — | „ | — | — | 26·5 | 31·0 | 21·0 |
| — | „ | — | — | 26·7 | 31·0 | 21·0 |
| — | „ | — | — | 26·1 | 29·0 | 22·0 |
| — | „ | — | — | 27·3 | 32·0 | 23·0 |
| — | Camel | — | — | 22·4 | 28·0 | 18·0 |
| — | „ | — | — | 24·0 | 27·0 | 20·0 |
| — | „ | — | — | 23·4 | 28·0 | 19·0 |
| — | „ | — | — | 22·9 | 27·0 | 18·0 |
| — | „ | — | — | 22·6 | 27·0 | 18·0 |
| 184 | Horse | — | — | 26·2 | 28·0 | 22·0 |
| 184 | „ | — | — | 25·9 | 29·0 | 21·0 |
| 184 | „ | — | — | 25·0 | 29·0 | 18·0 |
| 184 | „ | — | — | 25·1 | 30·0 | 19·0 |
| — | „ | — | — | 22·9 | 27·0 | 20·0 |
| — | „ | Osmic acid | Giemsa | 25·3 | 29·0 | 18·0 |
| 14 | Dog | — | — | 25·2 | 28·0 | 22·0 |
| 1 | „ | — | — | 26·7 | 30·0 | 21·0 |
| — | „ | — | — | 24·7 | 29·0 | 21·0 |
| 2 | „ | — | — | 26·3 | 30·0 | 23·0 |
| — | „ | — | — | 25·7 | 28·0 | 24·0 |
| — | Guinea-pig (1) | Osmic acid | Giemsa | 24·5 | 27·0 | 22·0 |
| — | „ (2) | „ | „ | 24·4 | 29·0 | 21·0 |
| — | „ (3) | „ | „ | 24·4 | 27·0 | 22·0 |
| — | „ (4) | „ | „ | 24·6 | 27·0 | 22·0 |
| 166 | „ | — | — | 23·9 | 28·0 | 21·0 |
| 87 | „ | — | — | 27·4 | 34·0 | 21·0 |
| — | „ | — | — | 27·2 | 31·0 | 24·0 |
| 191 | „ | — | — | 23·5 | 27·0 | 19·0 |
| — | „ | — | — | 26·8 | 31·0 | 25·0 |
| — | Rat (1) | Osmic acid | Giemsa | 24·8 | 28·0 | 21·0 |
| — | „ (1) | „ | „ | 22·7 | 25·0 | 20·0 |
| — | „ (2) | „ | „ | 24·7 | 29·0 | 21·0 |
| — | „ (2) | „ | „ | 23·2 | 25·0 | 20·0 |
| — | „ | — | — | 24·1 | 29·0 | 21·0 |
| — | „ | — | — | 24·3 | 27·0 | 19·0 |
| — | „ | — | — | 24·3 | 28·0 | 19·0 |
| — | „ | — | — | 26·5 | 32·0 | 22·0 |
| — | „ | — | — | 26·6 | 29·0 | 24·0 |
| 131 | „ | — | — | 25·1 | 27·0 | 22·0 |
| — | „ | — | — | 23·4 | 26·0 | 20·0 |
| | | | | 24·9 | 34·0 | 18·0 |

*Contents of Cell.*—According to Sir John McFadyean, a large proportion of *Trypanosoma evansi* show no distinct granules in front of the nucleus, whereas the majority of *Trypanosoma brucei* do. Moreover, when granules are present in the former, they are, as a rule, not so large or so numerous or so deeply stained as they are in the latter. These differences are not distinct except

Table II.—Distribution in respect to Length of 820 Individuals of *Trypanosoma evansi.*

| Animal. | Microns. | | | | | | | | | | | | | | | | | Average length. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18. | 19. | 20. | 21. | 22. | 23. | 24. | 25. | 26. | 27. | 28. | 29. | 30. | 31. | 32. | 33. | 34. | |
| Elephant ...... | — | — | 1 | — | 1 | — | 3 | 2 | 1 | 2 | 3 | 3 | — | 3 | 1 | — | — | 27·0 |
| „ ...... | — | — | — | 1 | 1 | 1 | 2 | 1 | 3 | 3 | 2 | 5 | — | 1 | — | — | — | 26·5 |
| „ ...... | — | — | — | 1 | 1 | — | — | 5 | — | 6 | 3 | 1 | 2 | 1 | — | — | — | 26·7 |
| „ ...... | — | — | — | — | 2 | — | 2 | 3 | 3 | 3 | 6 | 1 | — | — | — | — | — | 26·1 |
| „ ...... | — | — | — | — | — | 2 | 3 | 2 | — | 2 | 2 | 5 | 2 | 1 | 1 | — | — | 27·3 |
| Camel ......... | 1 | 2 | 2 | 2 | 1 | 6 | 3 | 1 | 1 | — | 1 | — | — | — | — | — | — | 22·4 |
| „ ......... | — | — | — | 3 | 2 | 3 | 3 | 4 | 2 | 3 | — | — | — | — | — | — | — | 24·0 |
| „ ......... | — | 1 | 2 | 2 | 3 | 1 | 5 | — | 4 | 1 | 1 | — | — | — | — | — | — | 23·4 |
| „ ......... | 1 | 2 | 3 | — | 2 | 1 | 3 | 5 | 2 | 1 | — | — | — | — | — | — | — | 22·9 |
| „ ......... | 2 | 1 | 4 | — | 1 | 3 | 5 | — | 1 | 3 | — | — | — | — | — | — | — | 22·6 |
| Horse ......... | — | — | — | — | 1 | — | 1 | 5 | 2 | 7 | 4 | — | — | — | — | — | — | 26·2 |
| „ ......... | — | — | — | 1 | — | 2 | 3 | 3 | 1 | 5 | 2 | 3 | — | — | — | — | — | 25·9 |
| „ ......... | 1 | — | — | 1 | 1 | 1 | 4 | 1 | 5 | 3 | 2 | 1 | — | — | — | — | — | 25·0 |
| „ ......... | — | 1 | — | — | 4 | 1 | 2 | 2 | 3 | 2 | 4 | — | 1 | — | — | — | — | 25·1 |
| „ ......... | — | — | 3 | 5 | 1 | 2 | 4 | 1 | 3 | 1 | — | — | — | — | — | — | — | 22·9 |
| „ ......... | 1 | — | — | — | 2 | — | 3 | 4 | 3 | 2 | 4 | 1 | — | — | — | — | — | 25·3 |
| Dog ............ | — | — | — | — | 2 | 2 | 2 | 3 | 8 | 2 | 1 | — | — | — | — | — | — | 25·2 |
| „ ............ | — | — | — | 1 | — | 1 | — | 1 | 2 | 9 | 4 | 1 | 1 | — | — | — | — | 26·7 |
| „ ............ | — | — | — | 1 | 1 | 1 | 7 | 5 | 3 | — | — | 2 | — | — | — | — | — | 24·7 |
| „ ............ | — | — | — | — | — | 2 | 3 | — | 3 | 9 | 1 | 1 | 1 | — | — | — | — | 26·3 |
| „ ............ | — | — | — | — | — | — | 6 | 3 | 5 | 3 | 3 | — | — | — | — | — | — | 25·7 |
| Guinea-pig ... | — | — | — | — | 3 | 1 | 8 | 3 | 1 | 4 | — | — | — | — | — | — | — | 24·5 |
| „ ... | — | — | — | 3 | 1 | 4 | 1 | 6 | 1 | 2 | 1 | 1 | — | — | — | — | — | 24·4 |
| „ ... | — | — | — | — | 2 | 5 | 3 | 5 | 3 | 2 | — | — | — | — | — | — | — | 24·4 |
| „ ... | — | — | — | — | 3 | 1 | 6 | 4 | 3 | 3 | — | — | — | — | — | — | — | 24·6 |
| „ ... | — | — | — | 3 | — | 7 | 2 | 4 | 2 | 1 | 1 | — | — | — | — | — | — | 23·9 |
| „ ... | — | — | — | 1 | — | — | 1 | 1 | 2 | 5 | 5 | 2 | 2 | — | — | — | 1 | 27·4 |
| „ ... | — | — | — | — | — | — | 3 | 2 | 1 | 5 | 3 | 4 | — | 2 | — | — | — | 27·2 |
| „ ... | — | 1 | 1 | 1 | 1 | 6 | 4 | 2 | 3 | 1 | — | — | — | — | — | — | — | 23·5 |
| „ ... | — | — | — | — | — | — | — | 5 | 4 | 5 | 4 | 1 | — | 1 | — | — | — | 26·8 |
| Rat ............ | — | — | — | 1 | — | 3 | 3 | 7 | 3 | 2 | 1 | — | — | — | — | — | — | 24·8 |
| „ ............ | — | — | 3 | 3 | 1 | 5 | 6 | 2 | — | — | — | — | — | — | — | — | — | 22·7 |
| „ ............ | — | — | — | 1 | 3 | 2 | 2 | 4 | 4 | 3 | — | 1 | — | — | — | — | — | 24·7 |
| „ ............ | — | — | 1 | 1 | 6 | 1 | 7 | 4 | — | — | — | — | — | — | — | — | — | 23·2 |
| „ ............ | — | — | — | 1 | 3 | 3 | 4 | 7 | 1 | — | — | 1 | — | — | — | — | — | 24·1 |
| „ ............ | — | 1 | — | 2 | 2 | 1 | 3 | 4 | 4 | 2 | 1 | — | — | — | — | — | — | 24·3 |
| „ ............ | — | 1 | — | 2 | 2 | 1 | 3 | 4 | 4 | 2 | 1 | — | — | — | — | — | — | 24·3 |
| „ ............ | — | — | — | — | 1 | 1 | 2 | 2 | 3 | 5 | 4 | — | 1 | — | 1 | — | — | 26·5 |
| „ ............ | — | — | — | — | — | — | 2 | 3 | 3 | 5 | 6 | 1 | — | — | — | — | — | 26·6 |
| „ ............ | — | — | — | — | 1 | 3 | 4 | — | 8 | 4 | — | — | — | — | — | — | — | 25·1 |
| „ ............ | — | — | 1 | 3 | 4 | 2 | 2 | 5 | 3 | — | — | — | — | — | — | — | — | 23·4 |
| Totals ......... | 6 | 10 | 21 | 40 | 59 | 75 | 130 | 125 | 108 | 118 | 70 | 35 | 10 | 9 | 3 | — | 1 | |
| Percentages ... | 0·7 | 1·3 | 2·5 | 4·9 | 7·1 | 9·2 | 15·8 | 15·3 | 13·1 | 14·4 | 8·5 | 4·3 | 1·2 | 1·1 | 0·4 | — | 0·2 | |

in well stained preparations, and Giemsa or some other Romanowsky method is the best for the purpose.

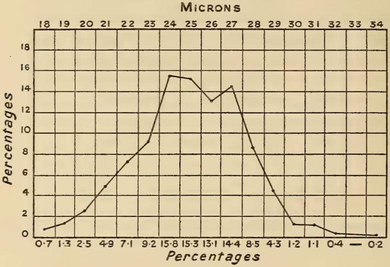
*Nucleus.*—Is oval or round and situated about the middle of the body.

*Micronucleus.*—Small and round, situated, on an average, 1·5 microns from the posterior end; maximum, 4, minimum, 0·5.

*Undulating Membrane.*—Is well developed and thrown into bold folds.

*Flagellum.*—Most of these trypanosomes have a free flagellum. It averages 4 microns in length; maximum, 9, minimum, 1. A small percentage are without free flagellum, but this, in my experience, is rare.

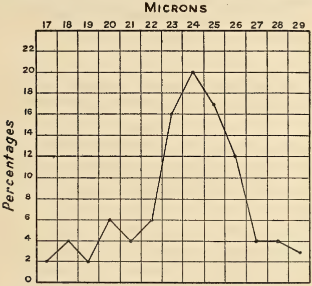
CHART 1.—Chart giving Curve representing the Distribution, by percentages, in respect to Length of 820 Individuals of *Trypanosoma evansi.*



COMPARISON OF THE DISEASE OF CAMELS, SUDAN, WITH THAT OF INDIA.

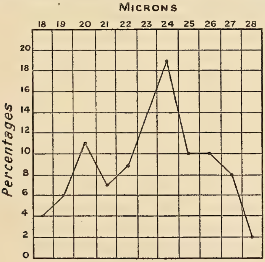
The disease of camels in the Sudan called Mbori is considered by Laveran and others to be caused by *Trypanosoma evansi.* It will therefore be interesting to compare a curve of the camel disease in India with the camel disease of the Sudan. I am indebted to Dr. Andrew Balfour, Khartoum, for a series of slides of this trypanosome, from which the following curve has been made:—

CHART 2.—Chart giving Curve representing the Distribution, by percentages, in respect to Length of *Trypanosoma evansi.* Sudan Camel Disease.



The next chart represents the curve of the Indian camel disease, and I am indebted to Mr. J. D. E. Holmes, M.A., D.Sc., Imperial Bacteriologist, Muktesar, India, for the slides from which the curve has been constructed :—

CHART 3.—Chart giving Curve representing the Distribution, by percentages, in respect to Length of *Trypanosoma evansi.* Indian Camel Disease.

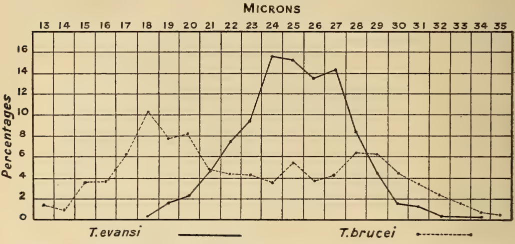


The similarity of these two curves is very striking, and affords some proof that the camel disease of India and that of the Sudan is caused by the same species of trypanosome.

### COMPARISON OF TRYPANOSOMA EVANSI AND TRYPANOSOMA BRUCEI.

Up to the present it has been usual to look upon *Trypanosoma evansi* and *Trypanosoma brucei* as being indistinguishable morphologically. It will therefore be interesting to compare the curves of these two species of trypanosomes.

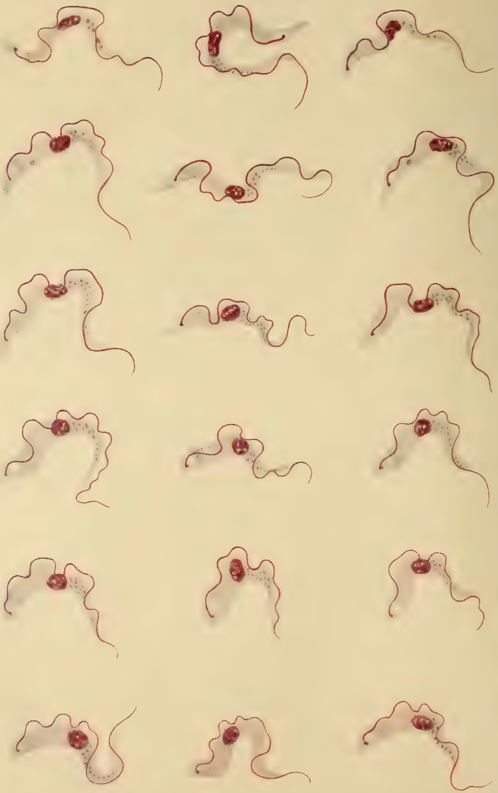
CHART 4.—Chart giving Curves representing the Distribution, by percentages, in respect to Length of *Trypanosoma evansi* and *Trypanosoma brucei.*



These curves are very unlike. It is therefore evident that if this method of recognising species of trypanosomes proves to be true, there ought in future to be no difficulty in separating these two species by this means alone. It might not always be possible to separate them by examining a single specimen of blood from each. Let us say a species of trypanosome is found in the Sudan, and it is a question of deciding whether it is *Trypanosoma brucei* or *Trypanosoma evansi.* It is inoculated into several animals—the horse, ox, monkey, dog, rabbit, guinea-pig, and rat. Two specimens of blood are made from each species on different days—40 trypanosomes from each species, 280 in all. If time were available, it would be better to measure a thousand. Then, if it is found that the curve lies mostly between 18 and 30 and not between 13 and 35, the diagnosis would be *Trypanosoma evansi.*

The average length of 172 *Trypanosoma brucei* was found to be 23·6 microns; that of *Trypanosoma evansi,* 24·9. It would, therefore, not be possible to separate these two species by length alone. Even if they are measured more minutely the result is the same.

For Table III, 180 *Trypanosoma evansi* and 91 *Trypanosoma brucei* have been measured and the average taken.



*T. Evansi*

*M. F. Bruce, del.* [illegible]

Table III.

| Species. | Posterior extremity to micro-nucleus. | Micro-nucleus to nucleus. | Length of nucleus. | Nucleus to anterior extremity. | Free flagellum. | Total length. |
|---|---|---|---|---|---|---|
| *Brucei*...... | 1·5 | 5·8 | 2·8 | 10·0 | 3·3 | 23·4 |
| *Evansi*...... | 1·5 | 5·9 | 2·5 | 10·4 | 4·0 | 24·3 |

Microns.

But, as we have seen, if a curve is made of the distribution of length among individuals of the species *Trypanosoma evansi* and compared with a similar curve of *Trypanosoma brucei*, then the difference between the two is striking.

## DESCRIPTION OF PLATE 5.

*Trypanosoma evansi* from the blood of various animals, fixed osmic acid, stained Giemsa. × 2000.

Note the similarity in appearance between this trypanosome and *Trypanosoma brucei*.* The nucleus is oval, or round, and is placed near the middle of the body. The micronucleus is small and round. One short and stumpy trypanosome, without free flagellum, is shown.

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* 'Roy. Soc. Proc.,' 1910, B, vol. 83, Plate 2.

# *On the Action of Senecio Alkaloids and the Causation of Hepatic Cirrhosis in Cattle.* (*Preliminary Note.*)

By Arthur R. Cushny, F.R.S.

(Received May 25,—Read June 15, 1911.)

(From the Pharmacological Laboratory, University College, London.)

The various species of Senecio in this country are generally regarded as harmless, the chief of them being the common ragwort and the common groundsel. In Nova Scotia, New Zealand, and South Africa they have, however, been associated with hepatic cirrhosis in cattle, which is known as Pictou, Winton, and Molteno disease in these countries. The species which induces this condition in Canada and New Zealand is apparently identical botanically with the common ragwort of this country, *Senecio Jacobœa*, while in South Africa the Molteno disease is associated with the *Senecio Burchellii* and the *Senecio latifolius*.

The symptoms of the disease are practically identical in these localities. The cattle are observed to be badly nourished for some time, but definite symptoms appear only three or four days before death, commencing in diarrhœa, dry and staring coat, and disinclination to feed. The cattle then lie down, or sometimes become frenzied and charge anyone who approaches. Soon coma and unconsciousness set in, and death follows.

The liver is found to present the appearance of chronic cirrhosis in some cases, in others there is marked venous congestion of this organ. The gall-bladder is distended with viscous, generally dark-coloured, bile, and there may be petechiæ in this organ, in the urinary bladder, and heart. The fourth stomach contains hæmorrhages and sub-mucous exudations. The intestine is inflamed around the openings of the bile ducts.

The disease being of great economic importance, a number of investigations have been instituted, which have proved that it is due to feeding on these species of Senecio.

With regard to the chemistry of the Senecio genus, Grandval and Sejour found two alkaloids in the common groundsel, which they term senecionine and senecine, and Watt found two others in the *Senecio latifolius* of Cape Colony, and has named them senecifoline and senecifolidine. These two bases were sent to me for pharmacological examination by Prof. W. R. Dunstan, and I have done a number of experiments with them, chiefly upon cats.

The symptoms induced are of two kinds, acute and sub-acute. The acute symptoms commence with nausea and salivation, extremely accelerated respiration, and, somewhat later, violent clonic convulsions under large doses. These acute symptoms generally pass off in the course of two or three hours, and the animal appears perfectly well very often for the next two or three days or longer. Some loss of weight may occur during this time, and then the sub-acute symptoms are introduced by a stool of rather loose consistency, loss of appetite, and in some cases vomiting. The animal then becomes weak and disinclined to move, and passes into a condition of apathy, stupor and coma, death following by failure of the respiration. These later symptoms succeed each other rapidly, death occurring within 24 to 48 hours after the first sub-acute symptoms.

Very similar symptoms were obtained in rats. The symptoms were the same whether the drug was given hypodermically or by the mouth. *Post-mortem* appearances varied a good deal in different animals. There was often found an unusual amount of fluid in the abdominal cavity, sometimes of a bright yellow colour. Small ecchymoses were sometimes found in the omentum, and fat deposits in the abdomen. The stomach contained black masses of half-digested blood, and the duodenum also contained some effused blood mixed with mucus. The liver was swollen and congested, and the gall-bladder was generally distended with very dark-coloured viscous bile, which could only be expressed from it with difficulty. Small hæmorrhages were often found in the lungs, pancreas, kidney, and some other organs.

Dr. C. Bolton kindly examined some of the organs microscopically and found marked congestion and hæmorrhages in the liver, the hæmorrhages being in most cases confined to the peripheral half of the lobules. The hepatic cells in the centre of the lobule were often normal, but further outwards they became distorted by the blood cells and stained badly, and towards the interlobular vein they were quite colourless and evidently in process of disintegration. Large areas of necrosis of the liver were found. In acute poisoning the liver cells often contained globules of fat. There was some infiltration of round cells round the portal canal, especially involving the smaller bile ducts and extending upwards from them between the liver cells. This feature was present in sub-acute cases, though it was more marked in chronic poisoning.

In chronic poisoning no symptoms, except loss of weight, were elicited until the drug had been given for over a month. The animal then died with the same appearances as in sub-acute poisoning. *Post mortem* the pyloric end of the stomach contained a quantity of black clotted blood, the duodenum had excessive mucous secretion, the liver was found in an advanced state of degeneration, most cells having disappeared and the few remaining staining badly. The greater part of the section was occupied by blood corpuscles in a state of decomposition. Round the vessels there were masses of round cells which appeared to be in process of change to connective tissue. The round cell infiltration extended also into the remains of the lobules and between the surviving liver cells. The cirrhosis had not proceeded so far as is described in cattle, but was of the same nature, and on the other hand was an obvious development of the process seen in animals which died from a single dose of the alkaloid.

The two alkaloids sent to me induced the same symptoms and the same changes, and seem to be equally toxic. The whole of the symptoms appear to arise from two different effects, one of them being an action on the central nervous system resembling that seen in many convulsive poisons, but this action is only induced by very large quantities. On the other hand, when smaller quantities are given, the dominating effect is hæmorrhage, which may occur in almost any organ, but which is constant in the liver and almost invariably present in the stomach and bowel. The hæmorrhage in the liver appears to be the cause of most of the other changes, such as the dropsy and jaundice, and the destruction of the liver cells appears to be the starting point for the cirrhosis. Together with the hæmorrhages in the stomach the hepatic changes may probably be the explanation of the loss of weight which forms a characteristic feature in chronic and sub-acute poisoning.

The results with the alkaloids of the *S. latifolius* suggested the examination of the action of the *S. Jacobœa* in this country. Inquiries in various parts of this country indicated that poisoning with this plant is unknown. In accord with this, I have been unable to obtain any symptoms from animals in which large quantities of the extract of the English ragwort were injected. On the other hand, the same plant growing in Canada has been shown to induce the characteristic cirrhosis, but an extract of a quantity of this plant grown in Canada also proved inactive. It is possible, however, that the plant from which my preparations were made had been collected at the wrong season, or the alkaloids may have undergone changes into some inert form in the course of preparation.

*S. silvaticus* collected in Yorkshire in August proved equally inactive. *S. vulgaris*, or common groundsel, collected in England and prepared in the same way, proved poisonous, the animals dying from symptoms resembling those arising from senecifoline, but with marked diarrhœa.

# *The Viability of Human Carcinoma in Animals.*

By Major C. L. Williams, M.D., I.M.S. (ret.).

(Communicated by Prof. C. S. Sherrington, F.R.S. Received June 14,—
Read June 29, 1911.)

(From the Cancer Research Laboratory, University of Liverpool—Mrs. Sutton Timmis
Memorial.)

The object of the present research is to determine the cell changes occurring in portions of human carcinoma implanted into animals, and more particularly to ascertain if such implanted tissues are capable of surviving for a time, and if so, the manner in which they succumb.

Implantation of human carcinoma into animals has been made by numerous observers, and the failure of such implantations to produce tumours is now an ascertained fact. Among the earliest experiments are those of Ballance and Shattock,* the objective of which was to determine if human carcinoma was transferable from man to animals; in these observations the immediate effect of implantation upon the cells of the growth was not determined. Von Langenbeck,† Jürgens,† Dagonet and Mauclaire,‡ and Gaylord§ produced in animals, by inoculation of carcinoma, tumours which, however, differed in structure from the original. Lewin‖ succeeded in obtaining inoculable granulomata in dogs by implantation of a human ovarian carcinoma, and also in rats by inoculation of a carcinoma of the cervix of the human uterus; this author made numerous attempts to obtain inoculable tissues by implantation of human carcinoma, but only the above two were successful.

The effect of implantation of mouse carcinoma and sarcoma into rats has been studied by Ehrlich,¶ who found that during the first 8—10 days after inoculation the rate of growth was scarcely less than before implantation;

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* "A Note on an Experimental Investigation into the Pathology of Cancer," 'Roy. Soc. Proc.,' 1890, vol. 48, p. 392; 'Brit. Med. Journ.,' 1891, 1, p. 565.

† Quoted by Lewin, *loc. cit.*

‡ "Versuche über die Übertragbarkeit des menschlichen Carcinoms auf die Ratte," 'Archives de Médecine Expérimentale,' 1904, No. 5, September.

§ "Über die Bedeutung der Plimmerscher (bezw. Sjobringschen) Körperchen und die durch menschliches Material erzeugtes Krebswucherung bei Tieren," 'Zeitschr. f. Krebsforschung,' 1903, vol. 1, p. 93.

‖ "Über Versuche, durch Übertragung von menschlichen Krebsmaterial verimpfbare Geschwülste bei Tieren zu erzeugen."

¶ 'Beiträge zur experimentellen Pathologie und Chemotherapie,' Leipzig, 1909, p. 135.

at the end of this period, however, growth ceased and gradual absorption occurred.

The method adopted in this research was that of implanting subcutaneously, with aseptic precautions, several selected portions of a malignant growth, freshly excised (pieces being put aside for microscopical observation), and following up the changes taking place in the cells of the growth by reference to sections of the implanted masses, removed for examination at different periods. The time elapsing between separation from the human body and the completion of implantation varied between 20 and 25 minutes. Implantation was effected by puncturing the skin with a straight cataract knife, and introducing on the point of the knife a piece of tissue having the form of a cube of 1—1½ mm. The portions of tumour implanted were removed at intervals of 1—13 days.

The number of tumours used for implantation was 41 ; 10 tumours were, however, employed in connection with unsuccessful implantations, so that only 31 need be referred to here. Of these, 19 were epithelioma (17 primary growths, 2 secondarily infected glands), 10 were scirrhus of the breast (9 primary growths, 1 secondarily infected gland), 1 was spheroidal-celled carcinoma, and 1 rodent ulcer. Implantation was made upon the monkey (20 tumours), rabbit (4 tumours), pigeon and guinea-pig (each 2 tumours), cat, rat, and mouse (each 1 tumour).

The usual causes of unsuccessful experiments were suppuration and failure to recover the pieces of tumour implanted. Suppuration was not met with so frequently as was expected, even in the case of growths ulcerated on the surface; when present, sometimes every piece of a tumour used for implantation became the seat of suppuration, in other cases suppuration occurred at some of the sites of implantation, while at others no inflammatory reaction occurred. A more serious difficulty was failure to recover the portion of tumour implanted. In a small number of cases this arose from the piece of tissue lying near the opening in the skin by which it was introduced, and in consequence desiccation ensuing. In other cases the piece of tumour implanted appeared to have moved from its original position and could not be traced. Not unfrequently the portion of growth implanted seemed to have undergone very rapid absorption, with the result that either the piece of tumour implanted could not be traced or no unmistakable growth of the original mass could be recognised in what is regarded as the remains of the implanted mass; difficulties of this kind were common from the fifth day after implantation onwards, and thus tend to limit the number of observations possible at later periods.

The result of implantation is summarised in the table. The usual course of events occurring after implantation was as follows :—In naked eye aspect the tumour, which was usually easily recognisable, being moist and greyish in aspect and lying loosely among the tissues of the host, did not appear much changed during the first two days. After this period it became dry and increasingly adherent to the surrounding tissues, from which it was less readily distinguished after the fifth day. On microscopic examination the central portions of the growth were found to have undergone necrosis during the first two to three days, presumably in part, at any rate, owing to defective supply of oxygen. Some of the cells at the periphery of the implanted mass, on the other hand, at first remained unchanged in appearance and exhibited more or less evidence of proliferation, generally presenting for the first two to four days mitoses, though fewer in number than was exhibited by the growth before implantation. After the fifth day all the cells of the implanted tumour had become altered and ceased to exhibit mitoses; their nuclear chromatin no longer presented the usual arrangement, but had become collected into irregular masses or fragments. Accompanying the necrotic changes occurring in the portions of tissue implanted, leucocytes, mostly polynuclear, made their appearance in large numbers, being replaced subsequently by mononuclear cells before which the remains of the implanted tissue disappeared. The disappearance of a piece of tumour of the form of a millimetre cube appeared to be completed after the second week of implantation.

In the summary given in the table the proportion of observations in which living cells and mitoses were noted during the first five days after implantation is given in a percentage form. It will be seen that inhibition of the function of cell division is early marked, while cell death occurs (at the periphery of the implanted mass) more slowly. If the experiments made upon the monkey alone are considered, the percentages obtained are more regular, being respectively: 67 and 25 on the first day; 69 and 8 on the second day; 67 and 0 on the third day; 37 and 12 on the fourth day; 20 and 20 on the fifth day. The experiments made with animals other than the monkey are too few in number to yield percentage values, but the circumstances that mitoses were not met with after implantation suggests that human carcinoma is less viable in these animals than in the monkey.

The result of implanting human carcinoma upon animals, it will be observed, is similar to that of implanting mouse carcinoma upon rats already referred to. In both cases a limited degree of inoculability is observable, some of the implanted cells continuing for a time to live and to divide, but whereas in the latter case the rate of proliferation is at first little affected and regression does not begin till the eighth to the tenth day, in the present experiments the

Table.—Implantation of Human Carcinoma. The + and − signs indicate the presence and absence respectively of living cells (l.) and mitoses (m.).

| No. of experiment. | Tumour. | Animal used for implantation. | Condition of implanted portions of growth at end of | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 day. | | 2 days. | | 3 days. | | 4 days. | | 5 days. | | 6 days. | | 7 days. | | 8 days. | | 9 days. | | 10 days. | | 11 days. | | 12 days. | | 13 days. | |
| | | | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. | l. | m. |
| 21 | Epithelioma of lower jaw... | Monkey | + | + | + | − | | | | | | | | | | | | | | | | | | | | | | |
| 22 | Epithelioma of floor of mouth | " | + | − | + | − | | | | | | | | | | | | | | | | | | | | | | |
| 23 | Epithelioma of tongue ...... | " | + | − | + | − | | | | | | | | | | | | | | | | | | | | | | |
| 24 | Epithelioma of prepuce...... | " | + | − | + | − | − | − | | | | | | | | | | | | | | | | | | | | |
| 29 | Epithelioma of fauces, glands secondary to | " | − | − | + | − | + | − | | | | | | | | | | | | | − | − | | | | | | |
| 31 | Epithelioma of jaw and tongue, glands secondary to | " | − | − | − | − | + | − | − | − | | | | | | | | | | | | | | | | | | |
| 32 | Epithelioma of cheek......... | " | + | − | | | + | − | − | − | − | − | | | | | | | | | | | | | | | | |
| 34 | Epithelioma of lower lip, glands secondary to | " | | | − | − | | | | | | | − | − | | | − | − | | | | | | | | | | |
| 37 | Epithelioma of tongue ...... | " | | | | | + | − | | | | | − | − | | | | | | | − | − | | | − | − | | |
| 38 | " " ...... | " | | | | | | | − | − | | | | | − | − | | | | | | | | | | | | |
| 18 | " " ...... | " | + | + | | | | | | | | | | | | | | | | | | | | | | | | |
| 27 | Scirrhus of breast ............ | " | + | + | + | + | − | − | + | + | | | | | | | | | | | | | | | | | | |
| 28 | " " ............ | " | − | − | + | − | − | − | | | | | | | | | | | | | | | | | | | | |
| 30 | " " ............ | " | + | − | | | + | − | − | − | | | | | − | − | | | | | | | | | | | | |
| 33 | " " ............ | " | | | | | + | − | + | − | − | − | − | − | | | | | | | | | | | | | | |
| 39 | " " ............ | " | | | | | | | − | − | | | | | − | − | | | | | | | − | − | | | − | − |
| 41 | " " ............ | " | | | − | − | | | | | − | − | | | − | − | | | | | | | | | | | | |
| 42 | " " ............ | " | | | + | − | | | + | − | | | | | | | − | − | | | | | | | | | | |
| 26 | Spheroidal-celled carcinoma of breast | " | − | − | − | − | | | | | − | − | | | | | | | | | | | | | | | | |
| 35 | Rodent ulcer of scalp ...... | " | | | + | − | | | | | + | + | | | | | | | | | | | | | | | | |
| 5 | Epithelioma of tongue ...... | Guinea-pig | | | + | − | | | | | | | | | | | | | | | | | | | | | | |
| 10 | Epithelioma of lip ............ | Rat | + | − | | | − | − | | | | | | | | | | | | | | | | | | | | |
| 12 | Epithelioma of ear............ | Rabbit | − | − | | | | | | | | | | | | | | | | | | | | | | | | |
| 15 | Epithelioma of temple ...... | " | − | − | | | | | | | | | | | | | | | | | | | | | | | | |
| 14 | Recurrent epithelioma below jaw | Pigeon | − | − | | | | | | | | | | | | | | | | | | | | | | | | |
| 16 | Epithelioma of tongue ...... | " | + | − | | | | | | | | | | | | | | | | | | | | | | | | |
| 16 | " " ...... | Mouse | + | − | | | | | | | | | | | | | | | | | | | | | | | | |
| 18 | " " ...... | Cat | + | − | + | − | | | | | | | | | | | | | | | | | | | | | | |
| 4 | Scirrhus of breast ............ | Guinea-pig | + | − | | | | | | | + | − | | | | | | | | | | | | | | | | |
| 7 | " " ............ | Rabbit | + | − | + | − | | | | | | | | | | | | | | | | | | | | | | |
| 9 | Scirrhus of breast, axillary glands secondary to | " | − | − | | | | | | | − | − | | | | | | | | | | | | | | | | |
| | Summary ......... | + ............ | 14 | 3 | 12 | 1 | 6 | | 3 | 1 | 2 | 1 | | | | | | | | | | | | | | | | |
| | | − ............ | 8 | 19 | 4 | 15 | 4 | 10 | 5 | 7 | 5 | 6 | 3 | 3 | 4 | 4 | 2 | 2 | | | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | per cent. ... | 64 | 14 | 75 | 7 | 60 | | 37 | 12 | 40 | 14 | | | | | | | | | | | | | | | | |

cells of the implanted portions of tumour ceased to proliferate at an earlier period, and were no longer living after the expiration of five days.

In conclusion I must express my indebtedness to Dr. Wakelin Barratt for the assistance he has given throughout the course of this work.

### *Summary.*

1. Portions of human carcinoma implanted into animals were observed during the first five days to retain their vitality and to exhibit mitoses after implantation.

2. After the expiration of this period no evidence of vitality was observed.

3. Mitosis was markedly inhibited within 24 hours of implantation, whilst the life of the implanted cells was abolished less rapidly.

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## *On Ceratopora, the Type of a New Family of Alcyonaria.*

By SYDNEY J. HICKSON, F.R.S., Professor of Zoology in the University of Manchester.

(Received June 15,—Read June 29, 1911.)

[PLATE 6.]

In the introduction to the British Museum Catalogue of the Jurassic Bryozoa (1896), Gregory remarks that, "to the palæontologist, who cannot check his conclusions by the evidence of vascular anatomy or embryology, these tube-dwelling animals are a vexation and a puzzle." This passage has reference to the difficulties that the palæontologist meets in determining the proper systematic position of many fossils that are known to us only by the tubular skeletons that they have left deposited in the rocks. Simple or colonial tubular skeletons, or more correctly shells, may be formed for the protection of recent sedentary animals belonging to the Protozoa, Cœlenterata, Annelida, Polyzoa, and Mollusca, and in many cases the only trustworthy guide to their systematic position is to be found in the study of the soft structures that formed the shell, the shell itself affording no distinctive characters.

In some cases the presence of septa, and in others of tabulæ, may indicate affinities; but even these characters may be misleading and give rise to erroneous conclusions. The presence of septa—now called pseudosepta—

in Heliopora led to the erroneous conclusion that Heliopora was a Zoantharian coral, and the presence of tabulæ in Millepora led to the classification of the Milleporidæ with other tabulate corals. It was not until Moseley examined the soft parts of Heliopora, and until Agassiz examined the soft parts of Millepora, that these corals were assigned to their proper position in the animal kingdom.

However, in the absence of soft parts to assist him, the palæontologist is obliged to base his classification on the skeletal structures, and consequently any new light that can be thrown on the structure and formation of the calcareous tubes of recent corals may be of considerable importance in his attempt to create a natural classification of the extinct forms.

The examination of an interesting dried coral obtained by the naturalists of the American steamer "Blake" has brought to light certain features which are, I believe, unique among tubular corals, and I have ventured to describe them in a separate paper, in the hope that they may be of service in solving some of the difficult problems of the fossil corals.

The single specimen of the species which I propose to call *Ceratopora nicholsonii* was obtained at the "Blake" Station 22, off Cuba, in 100 fathoms of water. Whether it was alive or not at the time of its capture I cannot say, but it was not preserved in spirit, and consequently nothing remains of its soft parts. It is undoubtedly the same species, if it is not actually the same specimen, as that figured by Agassiz in "The Three Cruises of the 'Blake,'" vol. ii, p. 83, but the only passage in the text that refers to it is as follows: "A supposed Favosites is probably a bryozoan genus, growing in the shape of a mushroom, and allied to Heteropora."

The specimen was forwarded to me for examination by Prof. Stanley Gardiner, together with some interesting letters from the late Prof. Alleyne Nicholson, addressed to Sir John Murray, on the subject of its structure.

The specimen consists of a lump of very hard crystalline limestone perforated in various directions by boring sponges, and projecting from the irregular mass of the lump there is a mushroom-shaped process (fig. 1, Plate 6) capped by a thin brown lamina, nearly circular in outline and 42 mm. in diameter, composed of small short vertical tubes. Without going into details, it may be stated that there can be little doubt that the whole lump of coral was formed by the successive growth of the organisms that formed the brown tubes of the cap, notwithstanding the fact that sections of the main substance of the specimen show no trace of tubular structure.

Before describing my own observations on the structure of the brown tubes, I may remark that Nicholson, in his letters to Sir John Murray, pointed out that the specimen differs from Heteropora in the absence of tabulæ, and in the absence of the pores by which the zooidal tubes are connected in that genus. Whilst hesitating to give any very definite opinion without more thorough investigation, he expressed his belief that the specimen is probably allied to the Helioporidæ.

When the surface of the cap is examined with a magnifying glass, it is seen to be pierced by a number of pores about 0·2 mm. in diameter (fig. 2). These pores are irregular in outline, but all of one kind. It is true there are some pores smaller than the majority, but there is nothing to suggest that the colony was dimorphic, or that anything corresponding with the mesopores of Heteropora were present. When seen in vertical fracture the pores are found to perforate the corallum to a depth of about 1 mm., but instead of being uniform in diameter, as they usually are in tubular corals, they rapidly narrow from above downwards and end abruptly in a blunt conical depression (fig. 3).

FIG. 3.

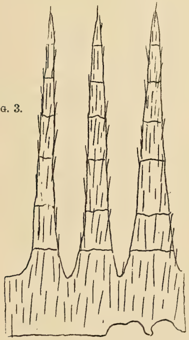

FIG. 4.

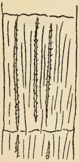

FIG. 5.

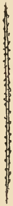

FIG. 3.—Diagram to illustrate shape of tubes of Ceratopora with the long needle-like spicules imbedded in the crystalline corallum. The transverse lines probably indicate lines of fracture.

FIG. 4.—Portion of one of the walls showing spicules imbedded in crystalline corallum. More highly magnified.

FIG. 5.—One of the spicules isolated, showing the small tubercles with which it is ornamented.

The tubes do not communicate with one another below the surface, and there are no tabulæ.

The walls of the tubes are brown at the surface, but this brownness gradually fades away, as the walls are traced downwards, into a pure white marble colour. This difference in colour is due, I believe, to a difference in chemical constitution as the walls grow older and thicker.

An examination of the vertical fracture further shows, when it is highly magnified, a number of long and very slender tuberculate spicules, partly imbedded in the walls and partly projecting on the surface and into the cavities of the tubes. All these spicules are arranged vertically, that is to say, parallel with the long axis of the tubes (figs. 3 and 4), and they project upwards into the cavity of the tubes as the latter widen out towards the surface. When a group of two or three tubes are broken off and placed in dilute nitric acid, the free projecting parts of the spicules rapidly dissolve ; the lower parts of the walls of the tubes also dissolve in the course of a few hours, but the upper, free, and brown parts of the tubes remain for several days as a soft flexible substance, in which the basal parts of the spicules may be seen until they are dissolved.

My interpretation of this experiment is that the walls of the tubes, as they were formed at the surface, were composed of a horny organic substance, in which a few long spicules of calcium carbonate were imbedded ; in the lower and older parts the horny substance became impregnated with calcium carbonate, and finally, at the base, nearly the whole of the horny organic substance became replaced by the inorganic salt.

The method of formation of the crystalline calcium carbonate is not very easy to understand, and, the specimen being unique and of small dimensions, I have not felt justified in making more than a few sections and other preparations. From these, however, I feel satisfied that the construction of this corallum is on very similar lines to that of the corallum of Heliopora as described by Bourne.* There are vertical trabeculæ from which the crystalline rods diverge in three directions, meeting in sutural junctions with similar diverging systems. These vertical trabeculæ can be traced for some distance down into the solid subjacent parts of the cap. There are no dark lines or centres of calcification such as occur in the Madreporaria. On crushing a very small fragment it breaks up into short irregular angular rods, very similar to the fragments of Heliopora drawn by Bourne in his fig. 24. From the consideration of these observations it seems quite probable that, as in Heliopora, the corallum of Ceratopora is formed by "crystallisation of carbonate of lime in an organic matrix."

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* 'Quart. Journ. Micr. Sci.,' 1899, vol. 41, p. 499.

From the evidence afforded by one of his letters, Nicholson appears to have noticed the spicules, but he considered them to be adventitious. If they are adventitious they belong to an Alcyonarian or possibly to a sponge that is unknown. No such spicules as these have yet been described. The arrangement of the spicules and their distribution in the walls of the tubes, however, give no support to the view that they are adventitious. If they were adventitious in the sense that the siliceous spicules of Polytrema and other Foraminifera are adventitious, we should expect to find them irregularly arranged and more numerous in some parts of the colony than in others.

If they are the products of the Ceratopora itself, as I believe they are, then we have another and most convincing proof that the genus is not related to Heteropora and the Polyzoa. The presence of tuberculate spicules of calcium carbonate suggests at once that Ceratopora is an Alcyonarian, and if it is true that, at the surface, these long spicules are imbedded in a horny organic substance, the condition is reminiscent of the walls of *Clavularia* (*Hicksonia*) *viridis*, in which long slender tuberculate spicules are associated with a number of horny fibres in the mesoglœa.

The principal difference between the spicules of Ceratopora and those of Hicksonia is one of size. It is difficult to determine the exact length of any one of the spicules of Ceratopora, as the part that is imbedded in the wall is difficult to trace, but their total length cannot be more than 0·3 mm. and their greatest diameter 0·01 mm. The spicules of Hicksonia, on the other hand, are 2·3 mm. in length by 0·18 mm. in diameter. The very small size of the spicules of Ceratopora is correlated with the very small size of the zooids that formed them, and the small size of the zooids may be regarded as one of the principal difficulties that may be felt in accepting the view that Ceratopora is an Alcyonarian.

The following list gives the diameter of the zooids of a few Alcyonaria for comparison with that of Ceratopora:—

| | mm. |
|---|---|
| *Hicksonia viridis* .................. | 3 |
| *Sarcodictyon catenata*............... | 1·5 |
| *Heliopora cœrulea* .................. | 0·75 |
| *Xenia novæ britanniæ* ............ | 0·8 |
| *Ceratopora nicholsonii* ............ | 0·2 |

The small size of the tubes of Ceratopora is not a character that, by itself, is sufficient to separate the genus from the Alcyonaria, and, taking into consideration all the other characters, the conclusion must be arrived at that the affinities with the Alcyonaria are more pronounced than with any other group of animals.

If Ceratopora is an Alcyonarian, it is necessary to consider what position it should occupy among the orders of its sub-class.

The long, isolated spicules do not afford a satisfactory character for the determination of its affinities, and although the spicules of one of the Stolonifera have some resemblance in arrangement and shape to the spicules of Ceratopora, this resemblance can be regarded only as an example of convergence. The massive crystalline skeleton in which the spicules are imbedded seems to indicate close affinities with Heliopora, the only recent Alcyonarian in which such a type of skeleton occurs. It is possible, of course, that this type of skeleton may have arisen independently within the sub-class, as we have examples of non-spicular calcareous skeletal structures in the axis of the Gorgonellidæ and in the axis of some of the Pennatulacea among recent Alcyonaria, and possibly also in the thecal walls of Syringopora, Favosites, and the Heliolitidæ among the fossil corals that are supposed to have Alcyonarian affinities.

But this type of skeletal structure, combined with the fusion of the thecal walls to form a honeycomb arrangement of the tubes, may be regarded as sufficient to justify the inclusion of Ceratopora in the order Cœnothecalia, to which Heliopora belongs. Nevertheless, Ceratopora differs from Heliopora in many important respects, and of these the most interesting is the presence of spicules, for in this respect the genus may be regarded as intermediate between the Cœnothecalia and the Stolonifera.

The monomorphic condition of the pores, the absence of tabulæ, and the complete closure of the tubes below by the continuous growth in thickness of the thecal walls, are further characters of importance that separate the two genera. On these grounds Ceratopora must be regarded as the type of a new family of Cœnothecalia, which may be defined as follows:—

### *Ceratoporidæ. New Family.*

Cœnothecalia forming a massive skeleton of crystalline calcium carbonate, in which a few slender spicules are imbedded. No tabulæ, the tubes closing below by the continuous growth of the thecal walls. Pores monomorphic and small (in the type species 0·2 mm. in diameter).

*Ceratopora nicholsonii,* new genus and species.—Off Cuba, 100 fathoms.

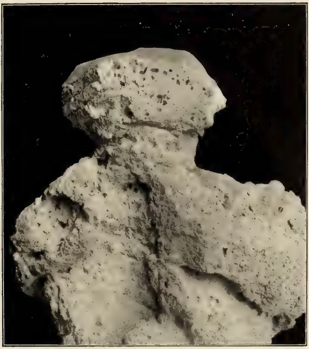

FIG. 1.

*Ceratopora nicholsonii.*—Side view of the mushroom-shaped process capped by the system of short tubes. Nat. size.

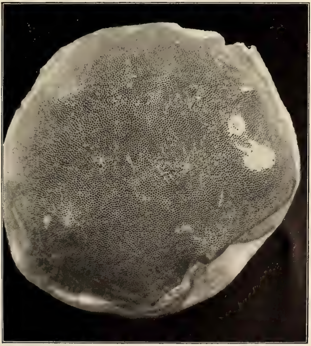

FIG. 2.

Surface view of the cap of Ceratopora, showing the pores. × 2 dias.

# *On Reflex Inhibition of the Knee Flexor.*

By C. S. Sherrington, F.R.S., and S. C. M. Sowton.

(Received and read June 29, 1911.)

(From the Physiological Laboratory, University of Liverpool.)

## 1. Introduction.

Study of reflex inhibition has been prosecuted more with extensor centres than with flexor. In the case of these latter, the experimental examination of the inhibition is of necessity somewhat differently circumstanced than in the case of the extensors. For both there is requisite a suitable background of reflex excitement against which inhibition may be evident. With the extensors this reflex background of excitement can be provided by postural tonus, and such tonus is readily obtained by use of the decerebrate preparation. With the flexors there is at present no procedure available for providing such tonic preparations. Recourse has to be taken to the production of reflex excitation of the centres by artificial stimuli applied to some appropriate afferent channel.* The background of contraction of the flexor muscle against which inhibition can become apparent is thus obtained by more artificial means. This latter procedure has its drawbacks; the background of reflex excitement it provides is less mild and less enduring than that furnished by natural tonus, and it is less enduring exactly in those approximately milder degrees which are particularly favourable for the manifestation of inhibition. On the other hand there are compensations, one being the more complete and rapid variation of the background in regard to its medium and higher intensities.

## 2. Method Employed.

As muscles typical of the flexor class we have chosen for our observations *semitendinosus* and *sartorius* (cat). These have been shown† to engage regularly as flexors, of knee and hip respectively, in the nociceptive flexion reflex of the limb, in the reflex step in its flexion phase, and in the scratch-reflex. Each of these muscles is readily prepared for the myograph by detachment of the lower tendinous insertion and liberation of the whole distal half of the muscle, the nerve and blood supply which enter above remaining intact. To immobilise the preparation the procedure has been as follows:—(1) Nerves severed: peroneal, popliteal, small sciatic, femoralis, obturator, external cutaneous and hamstring nerves of both limbs, with the exception in the case of the semitendinosus preparation of the branch to that muscle from the hamstring nerve of one limb, and in the case of the sartorius preparation of the branch to that muscle from the femoralis nerve of one limb. (2) Muscles resected: glutei tensor fasciæ femoris, psoas and psoas parvus, and all muscles attached to the femoral trochanters and intertrochanteric line. (3) The animal lying supine, with hips and knee semi-flexed, steel drills are inserted into the innominate bone, the outer femoral condyle and the lower end of tibia in both limbs; these drills are then clamped to heavy immovable uprights on the experiment table. These steps, as well as the whole of the preceding decerebration, are carried out under deep chloroform narcosis. For registration of the results a thread from the freed muscle tendon is carried over a light running pulley to a horizontal myograph. The tension of a light spiral spring is arranged to stretch the muscle to about its ordinary resting length. For stimulation of the afferent nerve or nerves we have employed faradism, or series of brief constant currents of alternating direction given by a v. Kries rotating rheonome* fed by four Leclanché cells, a graduated 100 ohms resistance box being in the main circuit. The electrodes have been non-polarisable, either of the du Bois-Reymond clay pattern or of the Utrecht pattern devised by Noyons.

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* Sherrington, 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 251.

† Sherrington, 'Journ. Physiol.,' 1910, vol. 40, p. 28.

The general plan adopted for the examination of the reflex effect of any particular afferent upon the flexor centre consisted in throwing that centre into reflex excitement as documented by contraction of the flexor muscle attached to the myograph, and then, while that contraction was in progress, stimulating the afferent nerve whose special influence on the centre it was desired to observe. This latter stimulation may be termed the intercurrent stimulation; the stimulation which provides the background contraction, against which the effect of the intercurrent stimulation has to show, may be termed the background stimulation.

## 3. Results.

### i. *Influence on the Knee Flexor (semitendinosus) exerted by Afferent Nerves of the Contralateral Hind-limb.*

The afferent nerves tested have been contralateral peroneal and popliteal, either separately or both together. The background excitation has been provided by stimulation of the corresponding nerves of the ipsilateral limb.

The effect of the contralateral afferent as thus tested is preponderantly inhibition. This preponderance of inhibition is very great. It holds for a wide range of intensities of stimulation. Its degree may be sufficient to entirely efface all trace of the background contraction. The inhibition is stronger the stronger the intercurrent stimulus (fig. 1, *a*, *b*, *c*), but it results in many cases from even quite weak intensities of stimulus.

With weak intensities of contralateral stimulus the effect is, however, not always inhibition. Such stimulation quite frequently causes contraction, *i.e.* augments the intensity of the contraction (fig. 2, *a*, *c*; fig. 3, *b*). The amount of contraction which it provokes is never in our experience large, although quite distinct and unmistakable. The contraction which weak stimulation of the contralateral afferent thus produces tends, while the stimulation is in progress, to subside and be replaced by inhibitory relaxation.

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* R. Metzner, 'Archiv f. Physiologie,' 1893, Supplement-Band, p. 84.

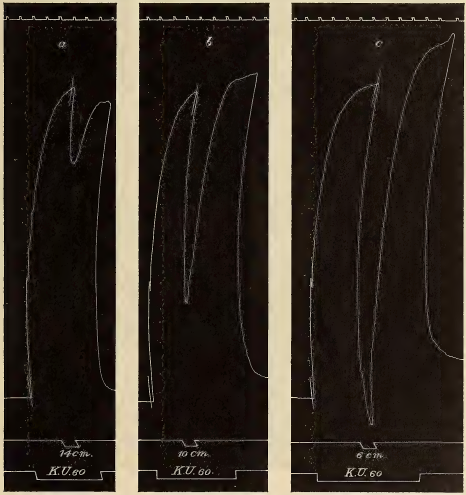

Fig. 1.—Inhibitions of the knee flexor, *semitendinosus* (cat, decerebrate). Lower signal marks stimulation (faradic) of ipsilateral afferent (peroneal + popliteal) exciting reflex contraction of the muscle. This stimulus remains of the same intensity, namely, 60 units of the scale of the Kronecker inductorium, in all three of the successive observations *a*, *b*, and *c*. Upper signal marks stimulation (faradic) of the contralateral afferent (peroneal + popliteal); this intercurrent stimulus is stronger in *b* than in *a*, and in *c* than in *b*, the secondary coil being at 14 cm. in *a*, at 10 in *b*, and at 6 in *c*. Time, in seconds, above.

The result given by the intercurrent stimulus then is an initial contraction followed by an ensuent inhibition (fig. 3, *d*). As the strength of the stimulus is increased, the initial contraction becomes more brief and less ample, and the ensuent inhibition appears earlier and is more pronounced. By further increase of the stimulus, inhibition without any apparent contraction at all results. As the strength of stimulus is increased further still, the only further change in the reflex effect is that the pure inhibition becomes more prompt and more profound.

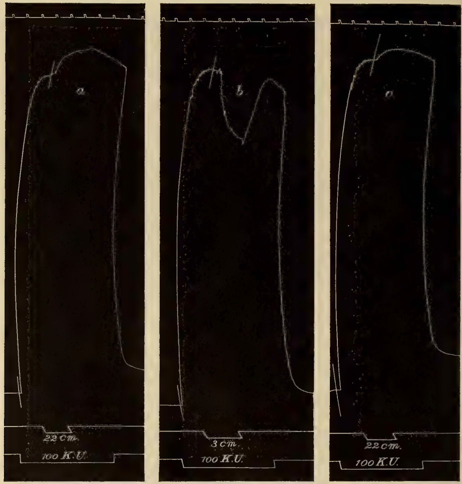

Fig. 2.—Reversal of reflex effect on increasing the intensity of the intercurrent stimulus, its result changing from contraction to inhibition. *Semitendinosus* (cat, decerebrate). Lower signal marks stimulation (faradic) of ipsilateral afferent (peroneal + popliteal) giving reflex contraction of the muscle. This stimulus remains of the same intensity, namely, 100 units, Kronecker inductorium, in all three of the successive observations. Upper signal marks stimulation (faradic) of the contralateral afferent (peroneal + popliteal); this intercurrent stimulus is quite weak (secondary coil at 22 cm.) in *a* and *c*, but in *b* is strong (secondary coil at 3 cm.). Time, in seconds, above.

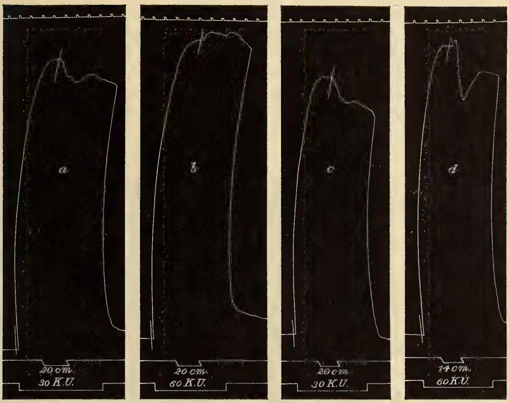

FIG. 3.—Influence of intensity of background-reflex on effect of intercurrent reflex. *Semitendinosus* (cat, decerebrate). Lower signal marks stimulation (faradic) of ipsilateral afferent (peroneal + popliteal). This stimulus in *b* and *d* is 60 Kronecker units ; in *a* and *c* it is weaker, namely, 30 Kronecker units. Upper signal marks stimulation (faradic) of contralateral afferent, and is of the same intensity, namely, secondary coil at 20 cm. for observations *a*, *b* and *c*, but for *d* it is stronger, namely, coil at 14 cm. In *a* and *c* the effect of the intercurrent stimulus is inhibitory, in *b* it is pressor, *i.e.* augmentative of contraction ; in *d* it is pressor also, but the increased contraction is followed by inhibition. Time above, in seconds.

### ii. *Influence of the Background Contraction on the Effect obtainable from the Intercurrent Contralateral Stimulus.*

It was shown in a previous communication that when two afferent nerves with mutually opposed influence on the same muscle are stimulated concurrently the effect on the muscle is an algebraic summation of the contraction and inhibition belonging to the two nerves respectively. Some of the examples then cited were furnished by the same muscle, semitendinosus, and the same afferents as chiefly employed in the present observations, and the present observations have confirmed the foregoing. They have also extended them in the following respect: Suppose a weak contralateral stimulus is chosen, such that it produces slight inhibition of a background contraction which is itself of rather weak intensity. If then the intensity of the background contraction be increased by stimulating the ipsilateral afferent more strongly, the inhibitory decrement produced by the contralateral stimulus becomes less, *i.e.* produces a shallower notch in the contraction myogram, in accordance with the above rule. If, however, the intensity of the background contraction be increased still further beyond a certain limit, which need not be very extreme, the effect of the intercurrent stimulation of the contralateral nerve is changed from inhibition to excitation, *i.e.* the contralateral afferent not only does not obviously diminish the contraction but augments it (fig. 3, *a* and *b*; fig. 4, *a* and *b*).

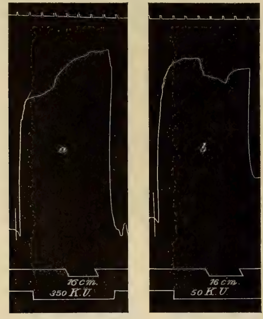

Fig. 4.—Decrease of background intensity changes the effect of a given intercurrent reflex from a pressor influence to a depressor. *Semitendinosus* (cat, decerebrate). Lower signal marks stimulation (faradic) of ipsilateral afferent (peroneal); the stimulus is more intense in *a* (350 Kronecker units) than in *b* (50 Kronecker units). Upper signal marks stimulation (faradic) of contralateral afferent (popliteal) and is of the same intensity in *a* and *b*. In *a* it augments the contraction, in *b* it decreases (inhibits) it. Time marked above, in seconds.

Besides intensity, other conditions also attaching to the background stimulation influence the effect of the contralateral nerve. In our experience the background of reflex contraction obtained by use of the brief alternating galvanic currents of the v. Kries rheonome for the ipsilateral afferent is more readily and amply inhibited by the contralateral afferent than is the reflex contraction furnished by ordinary faradism (fig. 5, *a*, *b*, *c*, *d*). Similarly, the inhibitory influence of the contralateral afferent is particularly easily and strikingly obtainable when pitted against the after-discharge contraction which frequently follows and prolongs, for a short time, a strong reflex after the strong stimulus which excited the contraction has been itself withdrawn. So also the inhibitory effect is markedly well obtained against the contraction elicited by a mechanical stimulus applied to the pinna of the ear. Occasionally in the decerebrate preparation the semitendinosus enters into somewhat prolonged reflex contractions whose source is not clear (fig. 6); and against these also the inhibitory effect of the contralateral afferent is extremely easily exerted.

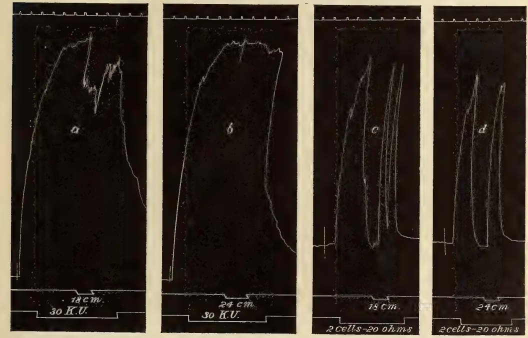

FIG. 5.—Similar intercurrent stimuli opposed to reflex backgrounds given by faradic and galvanic stimuli respectively. Lower signal marks stimulation of ipsilateral afferent by weak faradisation (30 Kronecker units) in *a* and *b*, by galvanic currents delivered by v. Kries rheonome in *c* and *d*. Upper signal marks weak stimulation (faradic) of contralateral afferent, secondary coil at 18 cm. for *a* and *c*, at 24 cm. for *b* and *d*. The inhibitory effect is more marked against the galvanic background. Time above, in seconds. *Semitendinosus* (cat, decerebrate).

And there is a further factor attaching to the background stimulation which likewise influences the effect of the contralateral afferent. When a given contralateral stimulus is repeated at intervals during the course of a prolonged reflex contraction, its inhibitory effect is greater in the later repetitions than in the earlier. This increase is regularly progressive and is often very marked (fig. 7, also fig. 6). It shows itself particularly when the reflex contraction is on the wane as judged by decline in the height of the myogram. It shows itself also when the intercurrent contralateral stimulus is repeated at a time when the reflex, as judged by the myogram curve, is exhibiting no marked decline, but remains as high, or almost as high, as it was at outset. It would seem, therefore, that, as the excitatory reflex proceeds, some central change ensues very soon after the reflex has reached its maximum, which renders the reflex discharge more and more open to inhibitory decrement. In other words, *fatigue* of the background reflex seems to favour markedly the operation of inhibition against the reflex.

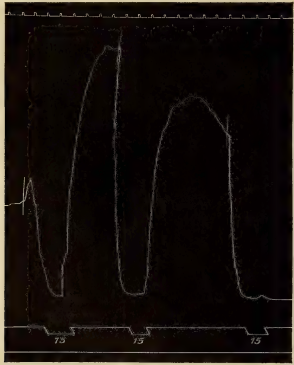

Fig. 6.—*Semitendinosus* (cat, decerebrate). At outset of the observation the muscle was already in contraction owing to some reflex the origination of which was not clear. The contralateral afferent (peroneal with popliteal) was then (left-hand notch of signal) faradised; this caused a transient increase of contraction, followed during the stimulation by marked inhibition, and succeeded, on withdrawal of the stimulus, by a rebound contraction. On repetition of this stimulus (middle notch on signal line), a marked inhibition again occurred, but without initial increase of contraction and followed after withdrawal of the stimulus by less marked rebound. On a third repetition (right-hand notch of signal line) the stimulus caused inhibition even more prompt than before, and no rebound contraction followed its withdrawal. Time marked above in seconds.

Further, when the contralateral afferent under a given stimulus of weak intensity produces the reflex augmentation of the background contraction

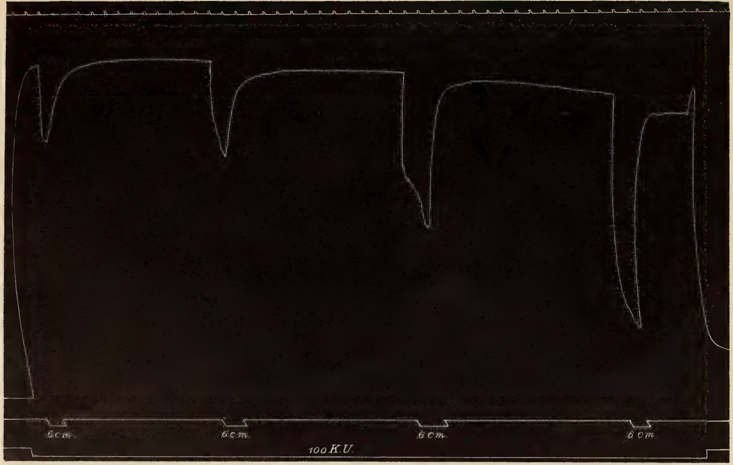

Fig. 7.—Increased inhibitory effect of a given stimulus during the progress of the background-reflex which it opposes. *Semitendinosus* (cat, decerebrate). Lower signal marks stimulation (faradic) of the ipsilateral afferent (combined peroneal-popliteal). Upper signal marks stimulation (faradic) of the contralateral afferent (combined peroneal-popliteal), this stimulus being of same intensity at all its applications, namely secondary coil at 6 cm. Time marked above, in seconds.

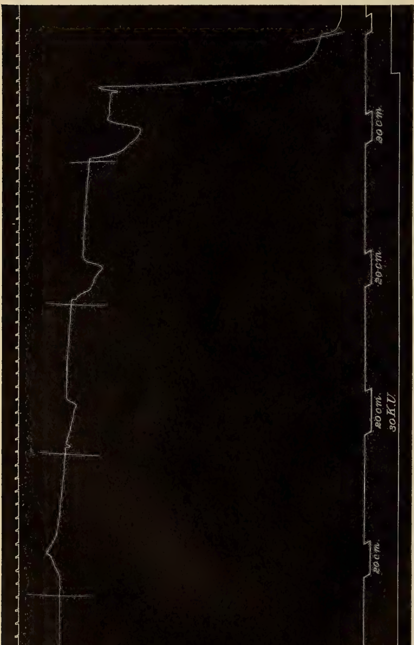

Fig. 8.—Reversal of reflex effect of a given stimulus with progress of the background-reflex. *Semitendinosus* (cat, decerebrate). Lower signal marks stimulation (faradic) of the ipsilateral afferent (combined peroneal-popliteal), intensity of stimulus being 30 Kronecker units. Upper signal marks stimulation of contralateral afferent (combined peroneal-popliteal), this stimulus being of the same intensity at all applications, namely secondary coil at 20 cm. Time marked above, in seconds.

which it often does, repetition of that same stimulus in the later course of the background contraction will produce inhibitory decrement of the background instead of excitatory increment (fig. 8, also fig. 6). In this case fatigue of the background ipsilateral stimulation actually reverses the reflex effect exerted by the contralateral afferent.

### iii. *Rebound.*

With the flexor muscle and centre, as with the extensor, the withdrawal of an inhibitory stimulus is frequently followed by a motor discharge from the centre and in result a contraction of the muscle. In our

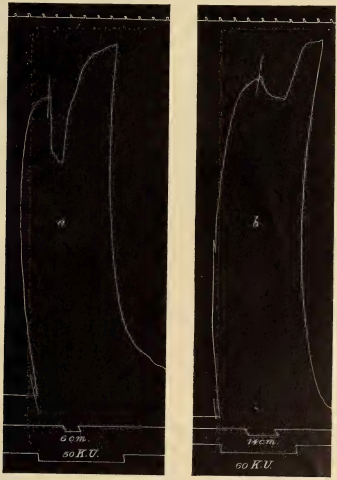

FIG. 9.—Rebound contraction. *Semitendinosus* (cat, decerebrate). Lower signal marks stimulation of ipsilateral afferent (combined peroneal-popliteal); upper signal marks stimulation of contralateral afferent (combined peroneal-popliteal). On withdrawal of the intercurrent inhibitory stimulus the reflex contraction caused by the ipsilateral stimulus increases to beyond the grade it had prior to the inhibition. Time marked above, in seconds.

observations, where the inhibitory stimulus is employed intercurrently against a background of contraction, this rebound manifests itself as increase of the background contraction to a height above that which it had prior to its depression by the intercurrent inhibition (fig. 9, also fig. 1, *c*, and fig. 6).

Circumstances favouring the exhibition of the flexor rebound are, just as in the case of extensor rebound, (1) considerable intensity of the intercurrent stimulus, (2) a somewhat brief duration of the intercurrent stimulus, (3) considerable intensity of the background stimulation.

Flexor rebound occurs not only when the background contraction is provided by electrical stimulation, but also when a "natural" reflex is in progress (fig. 6). Thus it follows intercurrent inhibition of a reflex contraction evoked by pinching the pinna of the ear, or when the reflex interrupted by the inhibition is of some source not clearly traceable in the experiment and arises apparently "spontaneously."

Marked rebound may ensue although the amount of elongation caused by the inhibitory stimulus may have been very small (fig. 10, *b*), owing to the length of the muscle at the time when the inhibitory reflex was evoked being already great. The flexor rebound in our experience does not present the prolonged tonic character which extensor rebound so often exhibits in the decerebrate preparation. The rebound contraction is short-lasting; when at all prolonged it frequently has a somewhat rhythmic form (fig. 10, *b*). In a series of somewhat quickly repeated elicitations it, like extensor rebound and even more markedly than that, diminishes rapidly. In other words, when provoked a number of times in rather rapid succession it soon tires out (fig. 6).

A point of interest in regard to the rebound is the following: As shown above, the contralateral nerve, although its predominant reflex effect on the flexor is inhibitory, does, under certain circumstances, produce instead of inhibition a weak contraction of the muscle. When this latter is its result, on withdrawal of the stimulus which has excited the weak contraction there not infrequently ensues increase of the contraction to beyond that already excited (fig. 10, *a*). In other words, rebound seems to ensue although the stimulus has excited no apparent precurrent inhibition. Possibly in these cases an inhibitory effect is really produced during the stimulus, but remains masked by concurrent excitation due to pressor fibres mixed with the inhibitory in the afferent nerve. On that supposition the rebound might still be post-inhibitory, although the inhibition was not apparent in the total result on the muscle.

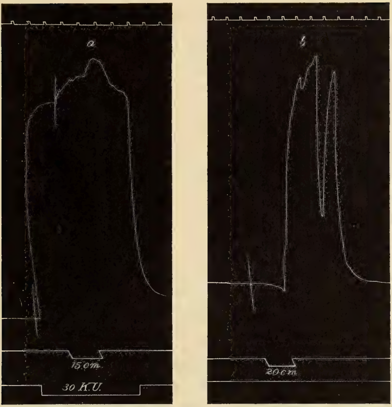

Fig. 10, *a.*—*Semitendinosus* (cat, decerebrate). Increase of intensity of the reflex background on withdrawal of a weak intercurrent stimulus whose only obvious effect had been pressor, not depressor. Lower signal marks stimulation of ipsilateral afferent; upper signal marks stimulation of contralateral afferent. Time marked above, in seconds.

Fig. 10, *b.*—Rebound after inhibitory stimulus which had, however, owing to toneless state of muscle, produced no relaxation of the muscle. *Semitendinosus* (cat, decerebrate). Upper signal shows stimulation (faradic) of contralateral afferent (combined peroneal-popliteal). The muscle at the time of application of this stimulus was resting and without apparent tonus. No obvious effect beyond questionable slight relaxations was caused by the stimulus during its application, but on its withdrawal there ensued immediately an ample though short-lasting rebound contraction. Time marked above, in seconds.

## 4. Conclusion.

Our observations show that the reflex influence of contralateral afferents (hind-limb) on the knee flexor resembles that of the ipsilateral afferents on the knee extensor.* In both cases moderate and strong stimulation produces reflex inhibition, while weak stimulation under certain conditions produces reflex contraction; and with stimuli of intensity belonging to a somewhat restricted range between weak and moderate the reflex effect is contraction followed by inhibitory relaxation. In these respects, therefore, both these sets of afferents conform with that type of afferent whose reflex reactions, as Fr. Fröhlich[*] has pointed out in the frog, are analogous to the reactions given by the nerve of the opening muscle of the arthropod claw. A paradigm of the results may be drawn up thus:—

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* Sherrington and Sowton, 'Roy. Soc. Proc.,' B, 1911, vol. 83, p. 435.

| Afferent limb-nerve. | Muscle. | Intensity of stimulation. | | | |
|---|---|---|---|---|---|
| | | Weak. | Intermediate. | Moderate. | Strong. |
| Ipsilateral ............... | Extensor | +[*] | + – | – | – |
| " ............... | Flexor[†] | | + | + | + |
| Contralateral ........... | " | +[*] | + – | – | – |
| " ........... | Extensor[‡] | | + | + | + |

+ signifies reflex contraction.  
– signifies reflex relaxation.  
+ – signifies reflex contraction followed during the stimulation by reflex relaxation.

[*] Under the circumstances mentioned previously in the text.  
[†] Sherrington, 'Journ. Physiol.,' 1910, vol. 40, p. 28.  
[‡] *Ibid.*

As briefly summarised in the above table it might appear that the sole factor determining whether, in these cases, reflex contraction or reflex inhibition ensued is the intensity of the stimulation. It was shown, however, in the more detailed descriptions supplied earlier in this and the previous paper[†] that that is really not the case. Another important determining factor appears to be the degree of the intensity of the reflex background at the time when the intercurrent reflex is tested.

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[*] F. Fröhlich, Verworn's 'Zeitschr. f. Allgem. Physiol.,' 1909, vol. 9, p. 55.

[†] Sherrington and Sowton, *loc. cit.*

# *The Structure and Physiological Significance of the Root-nodules of* Myrica gale.

By W. B. Bottomley, M.A., Professor of Botany in King's College, London.

(Communicated by Prof. J. Reynolds Green, F.R.S. Received June 21,—Read June 29, 1911.)

The peculiar nodule formations on the roots of *Myrica gale* were first described and figured by Brunchorst* in 1886, who stated that they were caused by an inhabiting fungus with septate hyphæ and terminal spores. Möller† in 1890 placed this fungus in the group Frankia, naming it *Frankia Brunchorstii*, and considered it to be closely related to a similar fungus in Alder nodules. In 1902 Shibata‡ stated that the fungus is found exclusively in a peripheral sub-cork layer of tissue, one to three cells thick, and because of its peculiar ray-branching and club-shaped spores, it belongs to the group Actinomyces. Peklo§ in 1910, working on greenhouse-grown plants, supported Shibata's view.

Roots of *Myrica gale* were obtained for this investigation from plants growing wild in Wales, Ireland, and the North of England, and from cultivated plants growing in the Chelsea Physic Gardens. In all cases the roots were found to possess nodules of varying size. The young nodules are from 2—3 mm. long and 0·8—1 mm. broad, but these by branching form "clusters," sometimes as large as a nutmeg, and surrounded by peculiar rootlets which grow out through the end of each nodule or branch. The branching is associated with the outgrowth of lateral roots, and is not due to dichotomy of the apical meristem of the root as is the case in the nodules of Cycas, Alder, and Elæagnus.

A transverse section of the tubercle shows a central tetrarch vascular cylinder similar to that of a normal root, and indicates that the tubercle itself is a modified root. The stele is surrounded by an endodermis characterised by neither radial dot nor thickened walls, but by the cells being filled with oil drops. Outside the endodermis are several layers of cortical cells covered on the outside with a definite small-celled cork layer. In mature nodules the cortical tissue is characterised by the presence of (1) somewhat enlarged cells filled with bacteria; (2) cells filled with oil drops. By means of Kiskalt's amyl Gram stain the bacteria can be seen *in situ* in the enlarged cells as small rods. Towards the apex of the nodule zooglea threads of bacteria are seen passing from cell to cell, comparable with the "infection threads" first seen by Marshall Ward in leguminous nodules.

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* J. Brunchorst, 'Unters. aus dem Bot. Inst., Tübingen,' 1886, vol. 2.

† H. Möller, 'Ber. d. Deutsch. bot. Ges.,' 1890, vol. 8.

‡ K. Shibata, 'Jahrb. f. Wiss. Bot.,' 1902, vol. 37.

§ J. Peklo, 'Cent. f. Bakt.,' 1910, (2), vol. 27.

The nodules arise as modifications of normal lateral roots. The cortical cells of a young root, before its emergence from the main root, become infected by bacteria. The normal growth of the root is thereby checked, but by division and growth of the cells containing the bacteria, the characteristic nodule with its tetrarch stele is formed. When the nodule has reached its full size, the end of the stele, surrounded by a few cortical cells, grows out from the apex of the nodule and forms a thin rootlet. Around this three branches or nodules (occasionally only two) arise endogenously as outgrowths from the cells surrounding the stele, repeating exactly the growth and structure of the primary nodule. By repeated branching in this manner the peculiar "cluster" nodules are formed.

No fungal hyphæ were observed in any of the young nodules examined, but "infection threads" containing bacteria were numerous, and it was evident that the formation of the nodules is caused by the action of the infecting bacteria.

Pure cultures of the bacteria from the cortical cells of the nodule were made. These on examination were found to be identical in structure and growth with the organism *Pseudomonas radicicola* found in leguminous nodules. They gave the characteristic staining reaction with aniline gentian violet and amyl alcohol, and formed colonies of oval shape on maltose agar.

Cultures of the bacteria were made in flasks with a solution containing 1 grm. maltose, 0·5 grm. potassium phosphate, 0·02 grm. magnesium sulphate in 100 c.c. water. After incubation for seven days at 25° C. nitrogen determinations of the culture solution gave the following results :—

| | |
|---|---|
| Control flask .................. | 0·53 mgrm. N per 100 c.c. |
| Inoculated flask ............... | 2·58 ,, ,, |

showing a fixation of nitrogen of 2·05 mgrm.

Young Myrica plants were obtained from Heysham Moss, some having nodules on their roots, others having none. Both kinds were planted out in a greenhouse in pots containing soil deficient in nitrogen. The plants without nodules did not thrive, and soon died, whilst those possessing nodules flourished and made a good growth.

It is evident from these experiments that the root nodules of Myrica are concerned with nitrogen assimilation, and that to the four families of non-leguminous plants—Alder, Elæagnus, Cycas, and Podocarpus—known to possess the power of nitrogen fixation by means of root-nodules, a fifth—Myrica—must now be added.

# *Note on the Surface Electric Charges of Living Cells.*

By W. B. Hardy, F.R.S., and H. W. Harvey.

(Received June 21,—Read June 29, 1911.)

The movement of free living cells suspended in a fluid through which an electric current is passing towards one or other of the poles has been described by many observers. In almost every case the movement has been observed in thin films of fluid under a cover-glass mounted in the way usual for microscopical examination. The cells do not always all move in the same direction; some migrate towards the anode, others to the cathode, and Thornton* found that in mixed suspensions of diatoms and amœbæ, or yeast cells and red blood corpuscles, the animal cells migrated to the anode, the vegetable cells to the cathode. He infers from this that animal and vegetable cells are oppositely electrified, the former being negative, the latter positive, to the fluid.

It is obvious at the outset that there are exceptions to this generalisation, for Becholt† describes a movement of bacteria towards the anode, the direction being reversed after agglutination. Dale‡ and Lillie§ also have described movements of animal cells to the cathode, but Thornton points out with some justice that in these cases the cells were not in their normal habitat.

The objection does not, however, apply to a natural culture of Gonium, Vorticella, and Amœba. In thin films such as Thornton used we found that the first two moved towards the cathode, while the amœbæ moved to the anode.

The movement of living cells, or indeed of any suspended particle, in films of liquid a millimetre or less in depth enclosed between glass plates is not open to the simple interpretation which Thornton places upon it. Arising from a contact difference of potential at the glass-water interfaces the upper and lower surface films of the water are dragged along in the electric field with considerable velocity on account of the ions they contain, and the flow along the boundary so produced is compensated under hydrostatic pressure by a return flow in the middle or round the edges of the stratum of water, if it be thick enough. The velocity of a particle past the observer is the sum of the velocity of the fluid and the velocity of the particle *through* the fluid, and since water is usually positive to glass and to particles suspended in it, the apparent velocity is commonly the sum of two velocities of opposite sign. Both movements, that of the water and that of the particle, are remarkably dead beat in thin films. If the bodily flux of fluid throughout the whole thickness under one field of the microscope were zero, and the stream lines were constant, the average velocity of particles taken through this entire thickness would, of course, give the mean velocity relative to the fluid.

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* 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 638.

† 'Zeit. Phys. Chem.,' 1904, vol. 48, p. 385.

‡ 'Journ. of Physiol.,' vol. 26, p. 219.

§ 'Amer. Journ. of Physiol.,' 1893, vol. 8, p. 273.

When a suspension of yeast cells and red corpuscles in isotonic sugar is observed in a U-tube wide enough to reduce the flux of fluid practically to zero, *both* migrate to the anode, but the corpuscles travel much the faster. In Thornton's experiment, therefore, the yeast cells move past the observer towards the cathode because they are unable to stem the current of water which is travelling in that direction. Yeast and blood cells under the conditions of the experiment are not oppositely electrified. Both are negative to the fluid, but the yeast cells migrate more slowly.

When the depth of the fluid is increased to 2·5 mm., the yeast and blood cells are seen to move in the same direction in the middle regions and in opposite directions in the film of fluid next the glass floor of the cell, and also in that next the surface.

Troughs of various shapes were used by us to observe these movements. Good results were got with one 2 cm. long, 3 mm. wide, and 2·5 mm. deep, with parallel glass sides, which opened at each end into a wide portion, divided into two compartments by a porous plate. The outermost compartment at each end was filled with a saturated solution of zinc sulphate, into which dipped electrodes of amalgamated zinc. The fluid was not covered in any way.

The current was between 0·0001 and 0·002 ampère, and was not allowed to run in the same direction for more than a few seconds at a time. With a current of more than 0·01 ampère, the vapour density rose to a point at which it deposited on the front of the objective—a remarkable result, which can be attributed only partly to heating. The image was, as a rule, completely "fogged" by dew when the current had run 4 seconds, the front glass of the objective being about 4 mm. above the surface of the fluid.

The difference in the apparent movement of red corpuscles and yeast cells in the layer next the glass, and in the middle of a stratum of liquid 2·5 or more millimetres deep, is not due to a difference in the interface between the cells and the fluid, for the velocity of one kind of cell with respect to the other was the same in both regions.

Countings of the number of divisions of a micrometer scale which contiguous yeast and blood separated from each other during a run of the current gave, for the layer next the glass, where they moved in opposite directions, 0·79 division per second, and for the middle region, where they moved in the same direction, 0·81 division per second—an agreement within the limits of error of observation.

Electrification of its surface, due to contact with the medium in which it lives, must modify endosmotically the passage of substances into or out of a living cell; one might expect, therefore, that a part of the work of the cell would be expended in controlling this polarisation. It is unfortunately difficult to get reliable information on this point.

The fact that yeast and blood corpuscles migrate to the anode in isotonic sugar at different rates probably means that the negative charge per unit area on the red corpuscle is greater than that on the yeast cell, for, according to theory, the velocity, due to shedding of the charged fluid layer, is independent of the size or shape of a particle, provided the slip at the interface be small compared with the dimensions of the particle.* This last condition is usually held to be fulfilled by solid particles of finite size, but it must be remembered that the interface between the enormous molecules of living matter and a fluid possibly differs widely in its properties from that between inert solid and fluid. Some features in the transport of fluid through living membranes seem to point to a very high coefficient of slip.

Another difficulty is that observation must be on cells in their natural habitat. Yeast and blood corpuscles in isotonic sugar solution are not in an indifferent medium, which leaves their properties unchanged. Isotonic sugar solution washes electrolytes out of muscle fibres, for instance, and so induces paralysis. The diffusion of salts out of yeast and blood corpuscles will polarise the surface to an extent determined by the osmotic properties of the surface and the nature of the salts.

The effect of poisons may be explained in this way. Chloroform, toluene, or traces of mercuric chloride reverse the sign of the charge on living cells, a second reversal, that is a return to the original charge, occurring after two or three days. The death change, however, is known to be accompanied by the liberation of salts, which previously were not "free,"† and the change in the polarisation of the surface may be referred to the diffusion of such salts out of the cell. The electrification of the surface certainly does not depend upon the intactness of the cell, for fragments of yeast cells broken up by pounding in a mortar moved in the same way, and at much the same rate, as did intact cells.

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* Lamb, 'Brit. Assoc. Report,' 1887, p. 501.

† Macdonald, 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 322; Macallum, *ibid.*, 1906, B, vol. 77, p. 165.

In spite of this, we incline to the view that the surface charge does vary with variations in the state of activity of the living cell, for in a natural mixed culture of Gonium, Vorticella, and Amœba, the fact that different cells of *the same species* migrated at different rates was very noticeable. The observations were made in the water in which the cells had been living, exposed to air, so as to leave the respiratory exchange normal. Red blood corpuscles are living cells, with very slight or no intrinsic chemical activity. In correspondence with this, they were found to migrate in blood serum to the anode at a remarkably uniform rate.

*Contact Potential at the Free Surface of Water.*—When finely powdered graphite was sprinkled upon distilled water contained in the observation cell already described, and the current, led through non-polarisable electrodes, was not more than 0·002 ampère, the following phenomena were noticed:—Of the graphite particles some broke through the surface of the water and sank slowly, others floated unwetted; the latter therefore served as an index of the movements of the actual skin. Except near the upper and lower surfaces the graphite particles migrated to the anode, just *below* the free surface and just above the glass they migrated to the cathode. The unwetted floating particles either did not migrate at all, or performed relatively slow irregular movements, which were not reversed on reversing the direction of the current and were due to heating. The movements of the particles contained within the water were dead beat, and reversed with the current. We may take it (1) that the actual surface skin is not propelled at all, or so slowly that the movement escapes detection in a period of, say, five seconds, during which submerged particles immediately below have hurried half across the field of view; (2) that the layer of fluid immediately below is driven by the field past this skin in the same direction and with the same order of velocity as the water past the glass. If additional proof of this were wanted, it is to be found in the fact that yeast and red blood corpuscles move in opposite directions in the layer immediately below the free surface, just as they do in the layer next to the glass, and for the same reason, namely, because the more slowly migrating yeast cells are unable to stem the current of water.

The stationary layer is exceedingly thin. With oc. 4, ob. B, focussed on the floating graphite, submerged particles showing rapid movement are scarcely out of focus, and the spectacle produces a remarkable impression of the presence of a tenacious skin which has sufficient rigidity to act as a relatively fixed layer past which the subjacent water is being driven.

The flow of water in electric endosmose is due to "relatively enormous electric forces acting on the superficial film, and dragging the fluid (as it were) by the skin through the tube."* At the free surface of a fluid, therefore, there must be relatively† enormous forces dragging the surface skin and the water in opposite directions if the movement of the water be due to a difference of potential between it and a surface film of impurities condensed from the air or neighbouring solids. The only escape from this conclusion is that the movement of the water is due to a circulation produced by the endosmotic movement of the layer touching the glass, but any compensating circulation would be opposed in direction to the flow at the glass face, whereas the surface flow is in the same direction—it is, in fact, precisely what it would be if the air and surface film were replaced by a plate of glass.

It seems difficult to avoid the conclusion that the film acts in the electric endosmose as though it were a rigid solid, and its properties are the same, when all ordinary precautions are taken to avoid contaminating the surface, as when a very thin layer of oil is allowed to spread over the water.

If the surface film really acts, as it would seem to, as a fixed layer past which the water is driven, since the stresses would be purely tangential, it is only necessary to regard it as having tenacity and as being anchored all round to the unwetted glass walls, and the apparent tenacity of the film will be partly true tenacity due to the forces between its component molecules and partly due to the work needed to rupture the film and expose a fresh water-air interface.

When the floating particles move at all, the movements are slight, irregular in direction (that is to say, they may be at an angle to the stream lines), and the direction is not reversed when the current is reversed. When the electrodes are placed directly in the distilled water, so as to cut out the large resistance of the end plates of porous earthenware, and the current thereby increased to 0·01 ampère or more, these movements are more rapid, and the submerged particles also now move in the same general direction as the floating particles, and their movement ceases to reverse when the electric field is reversed.

These movements, at first sight puzzling, admit of a very simple explanation. In the first place the direction is determined by the trough used and not by the current. That is to say, if the particles move from right to left no matter how the current is running, and the trough is displaced end for end, they now move from left to right. If we regard the gain of heat per unit of time from the current as being symmetrical with respect to the electrodes, the observed effects would be produced by an unequal *loss* of heat at the two ends of the trough, due to the disposition of the materials, to differences in their specific heat, or to asymmetrical conductivity of heat. The result would be an unequal rise of temperature in the two halves of the chamber, and consequent differences in surface tension. If this explanation be correct, though the direction of the movement of submerged particles is independent of the direction of the current, the velocity past the observer should vary. This was found to be the case.

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* H. Lamb, *loc. cit.*

† Relative, that is, to the surface stresses in ordinary flowing due to differences of hydrostatic pressure.

An analysis of the movements of the floating particles based on this hypothesis shows that in stronger fields the surface skin itself is dragged along. The following is an example:—Field approximately 35 volts per centimetre. Movement of floating particles always towards the right, but by reversal of current the velocity towards the cathode was 2,[*] towards anode 10. The drift due to heating therefore was 6, and the migration 4 divisions per second, and the latter was towards the anode. The surface film therefore was negative to the subjacent water.

## Appendix, *July* 26, 1911.

## *The Electrification of Surface Films.*

By W. B. Hardy, F.R.S.

The observations recorded in the preceding paper upon the endosmotic drift of the water in contact with a surface film involving foreign matter throw some light upon the range of molecular attraction. Under the conditions of the experiments, and for the short periods during which the current was on, it may be taken that there was no sensible hydrostatic pressure established due to change of level between the two ends of the trough. Under these conditions, if $u$ be the velocity of the water past the anchored surface film, we have

$$u = \frac{d\phi}{dx}\sigma\frac{1}{\gamma},\dagger$$

where $\sigma$ is the electric density, and $\gamma$ is a coefficient of sliding friction of water over the film.

The surface film acts as a thin sheet past which the fluid can flow, just as when the thickness of a soap film exceeds the range of molecular forces the interior mass may flow past the surface films which act as fixed boundary walls.

Whatever view be taken of the physical significance of the coefficient $\gamma$ it must be related in some simple way to the forces of attraction of the water for the superficial film. So long as the depth of the effective film is greater than the range of the molecular forces the attractive forces across the interface will be constant for films of the same composition and at the same temperature. When the thickness of the film is less than this range the Laplacian pressure at the interface, and therefore $\gamma$, must diminish and the velocity of the water under unit electric field increase.

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[*] Measured in divisions of the micrometer scale.

[†] H. Lamb, 'British Association Report,' 1887, p. 495.

The most probable assumption is that $\gamma$ varies directly with the intrinsic pressure at the interface.

Let the attraction of a molecule of water upon a molecule of the film be $mm'\phi(f)$, where $f$ is the distance between them. Then, if $z$ be the depth of the film and $dz$ an infinitely thin plate, the attraction of the whole mass of the water on the film is

$$2\pi m\rho\int_z^\infty \pi(f)\,f\,df, \quad \text{where} \quad \pi(f) = \int_f^\infty \phi(f)\,df.$$

The density of the water may be taken as uniform. The density of the film will vary rapidly. Call its density $\rho'$ and let

$$\psi(z) = \int_z^\infty \pi(f)\,f\,df.$$

The pressure at the interface will now be

$$2\pi\rho\int_0^z \rho'\psi(z)\,dz.$$

Leaving out of account for the moment the variation of density $\rho'(dz)$, and putting $\rho$ equal to unity, we have

$$p = 2\pi\int_0^z \psi(z)\,dz, \quad \text{which is equal to} \quad 2\pi\left[z\int_z^\infty \pi(f)\,f\,df + \int_0^z \pi(f)\,f^2\,df\right].$$

Putting $\pi(f) = \mathrm{K}\beta^{-1}e^{-\beta f}$ as an analytically simple hypothesis, this integral reduces to

$$p = 2\mathrm{K}\pi\beta^{-4}\left[2 - e^{-\beta z}(z\beta + 2)\right],$$

where $p$ is the pressure at the interface.

Rücker* gives as the range of molecular attraction 50 $\mu\mu$. The estimate is based upon measurements of the thickness of soap films made by himself in association with Reinold, and upon a critical analysis of measurements by Quincke and others. If $\beta$ be put equal to $10^6$ the force is approximately $\frac{1}{3}$ at 10 $\mu\mu$, $\frac{1}{150}$ at 50 $\mu\mu$, and vanishingly small at 100 $\mu\mu$. Thus $\beta = 10^6$ approximates closely to Rücker's estimate.

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* 'Journ. Chem. Soc.,' 1888, p. 222.

With this value, and on the assumption stated above, I find that the pressure at the interface would vary as follows:—

| Depth of film, in $\mu\mu$. | Per cent. of maximal pressure. |
|---|---|
| 50 | 95 |
| 40 | 90 |
| 30 | 80 |
| 20 | 66 |
| 10 | 45 |
| 5 | 25 |

The pressure would, however, not increase so rapidly as this with increase in the thickness of the film, owing to the variation of density in the film itself which is not taken account of in the above calculations. This is obvious when we remember that the film as it gains in thickness also gains in mean density owing to compression by the increase in the mean Laplacian pressure. The compressibility of the film will be relatively great since it is a transition layer between gas and fluid.

Turning to the observations themselves, the film was always so thin as to produce very slight effect upon the movements of shreds of camphor. This is what might be expected, since the distilled water was drawn from the bottom of a large glass reservoir, and all the chambers were thoroughly rinsed. From Rayleigh's measurements of such films* the thickness may be put with tolerable certainty as less than 2 $\mu\mu$—probably 1·5 $\mu\mu$.

At 2 $\mu\mu$ the interfacial pressure will be considerably less than 10 per cent. of its maximal value, and the coefficient of sliding friction $\gamma$ should have diminished proportionately.

Therefore, for the same potential gradient, the velocity of the water past the film should be much greater than it would be past a film 100 $\mu\mu$ thick, or past the glass if we assume that the layer of electric density at the glass-water interface does not differ widely from that at the film-water interface.

So much for theory. Observation shows that the velocity of the water past the surface film differs very slightly from that past the glass at the bottom of the trough. Thus a surface film of a thickness far below the accepted estimate of the range of molecular action acts like a mass of solid of, relatively, infinite thickness.

In considering this surprising result the three variables on which the relative velocity at an interface depends have to be remembered. The external electric field being taken as the same in all cases, they are: (1) the electric density at the interface, (2) the coefficient of sliding friction ($\gamma$), and (3) variations in the attraction of water for different substances.

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* 'Roy. Soc. Proc.,' 1890, vol. 47, p. 364.

Taking these in the order mentioned, so far as I know it the literature of electric endosmose without exception supports the view that the electric density on surfaces in contact with water varies within narrow limits. The velocity of a submerged visible particle is independent of size and shape, and varies directly with the electric density on the particle. It was easy in our experiments to see chance fragments, motes of dust, and living cells, travelling with velocities which agreed to within 1 or 2 per cent. The evidence, therefore, is in favour of the view that the electric density at the film-water interface did not differ much from that at the glass-water face.

By hypothesis $\gamma$ and $\phi(f)$, the coefficient of sliding friction and the intermolecular force, are dependent variables. If the thickness of the matter on each side of the interface exceeds the range of molecular attraction, $\beta$ varies directly as $\phi(f)$, where $\phi(f)$ refers only to the molecular attraction across the interface.

Here, again, there is evidence that $\beta$ does not vary. Putting the external electric field at unity, the velocity of a particle is given by the equation

$$V = -\sigma/\beta,^*$$

that is, in particles of 1 $\mu$ diameter and upwards, the velocity is independent of size and shape. But if $\phi(f)$ and therefore $\beta$ were different for different substances, the velocity should depend upon the nature of the particle.

Instead of this being the case we find proteïn masses, metals, and motes of dust in water, all moving in unit field with velocities of from 10 to $20 \times 10^{-5}$ cm./sec., and the variations within this range can be traced to the influence of the chemical nature of the particle upon the polarisation of the interface.

We are thus driven to the conclusion that the adhesion of the film to the water practically reaches its maximum when the thickness is still much less than the accepted value for the range of the molecular forces.

In the case of a small sphere at a potential different from the water urged along by an electric field, the hypothesis which has been adopted would make $\gamma$ sensibly constant until the diameter of the sphere fell to about 300 $\mu\mu$, when the pressure at the interface would be about 90 per cent.

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* Lamb, *loc. cit.*, p. 502.

of its maximal value.* This agrees with the fact that down to a diameter of 500 $\mu\mu$ the velocity still appears to be independent of size and shape.

It may be well, in conclusion, to emphasise the significance of the experiments. They seem to prove either that the coefficient of sliding friction between two phases is independent of the Laplacian pressure at the interface, or that the range of the molecular attraction is much less than Rücker's estimate—50 $\mu\mu$.

[P.S., *added July* 30.—During the present hot weather, when the water in the laboratory stands at 28° C., the film was found to have diminished in tenacity to a great extent. In order to give it the same degree of fixity under electrical stresses which it possessed at temperatures between 15° and 20°, it had to be thickened with oil until a blue film was produced, which almost entirely stopped the movements of camphor.]

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## *The Origin of Osmotic Effects.* IV.—*Note on the Differential Septa in Plants with reference to the Translocation of Nutritive Materials.*

By Henry E. Armstrong, F.R.S., and E. Frankland Armstrong.

(Received and read June 29, 1911.)

Our communication to the Society which was read on June 2 last year† was made under the primary title attached to the present communication, because it appeared to us that many of the osmotic phenomena in plants were to be correlated with effects produced initially by the class of substances to which we have ventured to extend the term *Hormone*, introduced by Starling but applied by him only to certain members of the group. The observations recorded were made with leaves of *Prunus laurocerasus*. In a more recent communication,* in which we have discussed observations made later in the year with leaves of *Aucuba japonica* and a number of other plants, we again called attention to the osmotic effects conditioned by hormones and the suggestion was advanced that the translocation of nutritive materials takes place periodically.

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* The integral for the pressure at the surface of a sphere in a vacuum is given by Rayleigh ('Phil. Mag.,' 1890 [2], vol. 30, p. 456), as $2\pi\int_0^{2r} f^2\pi(f)\,df - \frac{\pi}{f}\int_0^{2r} f^3\pi(f)\,df$. Putting $\pi(f) = \frac{K}{\beta}e^{-\beta f}$, this reduces to $\frac{\pi K}{\beta^2}\left[e^{-2r\beta}\left(4\frac{r}{\beta}+8\frac{1}{\beta^2}+6\frac{1}{r\beta^2}\right)-6\frac{1}{r\beta^3}+4\frac{1}{\beta^2}\right]$.

† "The Origin of Osmotic Effects. III.—The Function of Hormones in Stimulating Enzymic Change in Relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures," 'Roy. Soc. Proc.,' B, 1910, vol. 82.

Taken in conjunction with those made by Adrian Brown, our observations show that the outer differential septa in plants are permeable only by substances of a particular type—apparently only by substances having but slight affinity for water; consequently, if the argument apply to plant cells generally, ordinary nutritive materials, such as the sugars, for example, cannot pass through unless the septa are in some measure broken down. It almost stands to reason that the translocation of carbohydrates and many other materials must take place periodically: that at some times the cell walls must be permeable whilst at others impermeable. As we have already pointed out, Darwin's work on insectivorous plants appears to be full of evidence that such is the case.

It is abundantly clear from the behaviour of *Saxifraga sarmentosa*, for example, that the cells generally are lined with a septum which is differentially permeable. When placed in a solution of greater osmotic tension than that within the cells, the coloured fluid is retracted in the well-known way; this effect is easily reversed and the change may be brought about time after time provided that the membrane enclosing the cell contents remain uninjured. The effect cannot be produced after exposure to chloroform and there are many other substances which act similarly; it is therefore to be supposed that it is conditioned by the differential permeability of the thin protoplasmic membrane which lines the cell.

In the account of our experiments with leaves of *Prunus laurocerasus* we stated that, of the three substances into which the glucoside characteristic of the plant, prulaurasin, is resolved—glucose, benzaldehyde and hydrogen cyanide—the last two act as hormones, each being capable of conditioning hydrolysis. In studying the action of these and other hormones, as was to be expected would be the case, significant differences have been brought to light; we propose to make these differences the subject of careful study. On the present occasion we desire to call attention to the special effect produced by hydrogen cyanide, as this appears to us to raise issues of peculiar and wide significance.

If kept in water, leaves such as those of *Prunus laurocerasus* or of *Aucuba japonica* not only remain unchanged during many days but nothing diffuses out into the liquid; if a substance which can penetrate into the leaf be added to the water, as a rule, not only does the leaf change in appearance but substances soon pass out from it into the surrounding liquid. In the case of *Aucuba*, for example, an amount of reducing sugar equal to from 3 to 4 per cent. on the original weight of the leaf diffuses out into the solution in the course of three or four days.

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* "The Function of Hormones in Regulating Metabolism," 'Annals of Botany,' 1911, vol. 25, pp. 507—519.

If the hormone used be hydrogen cyanide, however, although changes take place within the leaf, no reducing sugar passes out into the solution. It suffices to use a solution containing only 0·2 per cent. of the cyanide. The difference has been noticed in the case of a considerable variety of leaves, in roots such as that of the radish and beet, in unripe fruits (cherry and currant) and in unripe seed pods.

Most leaves become coloured more or less distinctly brown, some even black, on exposure in water saturated with either chloroform or toluene; but in a solution of hydrogen cyanide the colour change is far less marked, the green colour being preserved often during a considerable period. The difference is particularly noticeable in the case of leaves which blacken in chloroform, such as those of *Vicia faba*, for example. The almost black colour assumed by the *Aucuba* leaf in presence of chloroform is evidently due to several superposed effects; in the cyanide solution such leaves become highly coloured but not nearly to the same extent as when they are exposed to the action of other hormones.

These differences would seem to be proof that differential septa which break down under the influence of most hormones remain intact when hydrogen cyanide is used, though hydrolytic changes take place within the leaf under the influence of this latter agent.*

Taking into account the manner in which leaves change in appearance when exposed in water saturated with a substance such as toluene, there can be little doubt that the coloration is at least mainly an oxidation effect; and bearing in mind what is known of the effect hydrogen cyanide has in inhibiting oxidation, it appears probable that differential septa remain intact because the "oxidase effect" is eliminated in presence of hydrogen cyanide.

It is well known that oxidation processes are at a maximum in plants during the period when light is inactive and that growth takes place chiefly during this period: the translocation of nutritive materials which necessarily sets in during this period may well take place because the septa are broken down and rendered permeable by oxidation; they may be repaired subsequently, when assimilatory processes become ascendant.

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* We have already called attention in our previous communication to the production of reducing sugars within the laurel leaf when it is exposed to the action of hydrogen cyanide.

We are extending our observations to animal tissues.

We have to thank Mr. Mummery for the assistance he has rendered to us in carrying out a number of the experiments.

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# *The Properties of Colloidal Systems.* III.—*The Osmotic Pressure of Electrolytically Dissociated Colloids.*

By W. M. Bayliss, F.R.S., Institute of Physiology, University College, London.

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CONTENTS.

| | PAGE |
|---|---|
| Hydrolytic Dissociation | 230 |
| Electrolytic Dissociation | 230 |
| The Factors of the Osmotic Pressure | 232 |
| Electrolysis of Congo Red | 240 |
| The Potential Difference at the Membrane | 243 |
| The Distribution of Salts between Solutions of Congo Red and Water separated by a Membrane | 249 |
| The Action of Carbon Dioxide | 251 |
| The Temperature Coefficient of the Osmotic Pressure of Congo Red | 253 |
| Summary of Conclusions | 253 |

In a previous paper[*] I showed that the osmotic pressure of solutions of Congo red, as measured directly in an osmometer with a membrane of parchment-paper, is about 90—95 per cent. of that which they should have if the dye were present as undissociated single molecules, such as those of glucose or urea. Attention was chiefly directed, in the paper referred to, to the fact that a body behaving as a colloid gives as high an osmotic pressure as if it existed in solution as single molecules and not as aggregates. It is to be remembered, however, that Congo red is the sodium salt of a fairly strong acid and as such must be dissociated to a considerable degree in solutions of the concentration employed. On this account, the interpretation of the experimental results required further work. Subsequent investigations have shown that there are many difficulties in the way of a satisfactory explanation.

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[*] 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 269.

As will be seen later, the close correspondence between the osmotic pressure found and that of the dye if undissociated must be due to the chemical nature of this particular dye as a disodium salt of a dibasic acid. Other dyes of a similar constitution, but of different sodium content, such as Chicago blue, do not show this property. I regard it as a somewhat unfortunate accident that Congo red was chosen as the object of the first investigation. Attention was thereby diverted from the more essential facts.

As it will frequently be necessary to refer to the osmotic pressure as it would be shown by a body present in solution in undissociated single molecules, I propose, for convenience, to speak of it as the "molecular" osmotic pressure, although of course the expression is not strictly correct.

### *Hydrolytic Dissociation.*

It is fortunate, as an initial simplification of the problem, that no trace of hydrolytic dissociation can be detected in solutions of Congo red. When such solutions are separated from water by parchment-paper, no free alkali diffuses out, such as happens, for example, from solutions of sodium oleate. In this result I find myself in agreement with other observers.* The acid of Congo red, in fact, behaves as a strong one, no doubt owing to the two sulphonic acid groups contained in its molecule. Its solutions attack metallic zinc. In its use as an indicator it is well known that the second sodium atom can only be displaced by strong mineral acids. It is evident that the $NH_2$ groups of the naphthylamine residues are practically neutralised by the sulphonic acids. The basic properties of this amino-acid are so weak as to be negligible. I have been unable, indeed, to find any evidence that it forms salts even with hydrochloric acid.

It is interesting to note incidentally that even sodium caseinogenate appears to be hydrolytically dissociated only to a minute degree. Roaf† makes the same statement with respect to the sodium salts of the serum proteins. Hardy‡ also found the hydrolysis of the sodium salt of globulin to be very slight.

### *Electrolytic Dissociation.*

Although, since my former experiments were made, measurements of the conductivity of Congo-red solutions have been published,§ I thought it best to determine that of the particular sample of dye used for the experiments to be described below. The dye was Kahlbaum's best preparation, but was found to contain appreciable quantities of sodium chloride and a small amount of sodium sulphate. It was therefore purified by "recrystallising" from dilute alcohol. The hot saturated solution on cooling deposited a considerable part of its contents. Although this deposit did not seem to be actually crystalline, it was possible to purify the dye in this way, naturally with considerable loss. After repetition of the process for five times, the conductivity of solutions of equal concentration from two successive treatments became identical, so that no foreign electrolyte was present. Several determinations of conductivity in successive dilutions were made and a curve made for future use in order to obtain the concentration corresponding to a known conductivity.

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* Pelet and Wild, 'Kolloid. Zeits.,' 1908, vol. 3, p. 174.

† 'Quart. Journ. Exp. Phys.,' 1910, vol. 3, p. 175.

‡ 'Journ. Physiol.,' 1905, vol. 33, p. 276.

§ 'Die Theorie d. Färbeprozesses,' von L. Pelet-Jolivet. Dresden, 1910, p. 27.

The following numbers (Table I) will serve as an example of an experiment:—

Table I.

| Dilution in litres. | Concentration in millimols. per litre. | Specific conductivity in recip. ohms × $10^6$. | Molar conductivity in recip. ohms × $10^4$. | Dissociation, per cent. |
|---|---|---|---|---|
| 14 | 71·4 | 6772 | 950 | 45·6 |
| 28 | 35·7 | 3782 | 1060 | 51 |
| 56 | 17·86 | 2086 | 1166 | 56 |
| 112 | 8·93 | 1154 | 1293 | 62 |
| 224 | 4·46 | 662 | 1482 | 71·2 |
| 448 | 2·23 | 367 | 1645 | 79 |
| 896 | 1·12 | 201 | 1800 | 86·5 |
| 4480 | 0·223 | 47 | 2083 | 100 |

The determinations were made at 25° C., the same temperature at which the osmotic pressure measurements were made. The dilutions were made in quantities of 40 c.c. in a series of flasks, as it was found that the usual method of removing solution from the conductivity vessel and replacing with water was apt to lead to inaccuracy with the more concentrated solutions, owing to their viscosity. Further dilution beyond 4480 litres gave only slightly increased values of the molar conductivity, and from curves it appeared that the limiting value at infinite dilution would be $2100 \times 10^{-4}$ recip. ohms, which was accordingly taken as the basis of calculation for the degree of dissociation of the various dilutions.

It will be noticed that, although the acid of Congo red is stronger than acetic acid, the degree of ionisation in the more concentrated solutions is less than that of sodium acetate. For example, in a dilution of 32 litres sodium acetate is dissociated to the extent of 86 per cent., whereas Congo red at the same dilution is only dissociated to 52 per cent. This is probably due to colloidal association of molecules in the latter case. If so, it presents an interesting case for investigation, since ionisation would be some function of the surface of the aggregates.

Owing to the fact that this dye is the disodium salt of a conjugated disulphonic acid, it is of interest to compare its conductivity with that of disodium sulphate. The molar conductivity of this latter at infinite dilution, under the same conditions as Congo red above, was found to be $2600 \times 10^{-4}$ recip. ohms. Sodium chloride, with a corresponding value of $1200 \times 10^{-4}$, and sodium naphthylamine-sulphonate with that of $1150 \times 10^{-4}$, may be compared.

From the values given it will be seen that in moderately dilute solutions, such as those whose osmotic pressure was given in my previous paper, Congo red may be considered to be a strong electrolyte. The migration velocity of the anion, although it has a molecular weight of 650, must be not much less than that of $SO_4'$. Biltz and v. Vegesack* calculate this migration rate as 57 in the same units in which $SO_4'$ is 67.

Congo red does not obey the Ostwald dilution law, the values of the "constant" showing steady and considerable diminution with increasing dilution.

It was found impossible to obtain at 25° C. solutions more concentrated than 1 molecule in 14 litres, or 4·975 per cent. At this dilution the salt was dissociated to the extent of 45 per cent., whereas sodium sulphate is dissociated to 74 per cent.

### *The Factors of the Osmotic Pressure.*

The experimental facts which have to be accounted for may be realised by taking the case of Congo red at a dilution of 120 litres. I find that the osmotic pressure of such a solution is slightly lower than what it would be if the salt were undissociated. But measurements of the electrical conductivity show that it is dissociated to the extent of at least 60 per cent. at this dilution, so that, if the dissociation is into two $Na^{\cdot}$ and one bivalent anion, the concentration of osmotically active elements within the membrane is $m/66$ in ions and $m/300$ in non-dissociated salt, or $m/54$ in all.

A simple explanation of the discrepancy has been advocated by Biltz and v. Vegesack,† viz., that the $Na^{\cdot}$ ions to which the membrane is permeable do not take any part in the production of osmotic pressure; so that, since only one anion is formed and the membrane is impermeable to it, the undissociated and dissociated fractions of the salt are equivalent in osmotic activity. On this view it is therefore immaterial whether the salt is or is not dissociated.

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* 'Zeit. f. physik. Chem.,' 1910, vol. 73, p. 481.

† 'Zeit. f. physik. Chem.,' 1910, vol. 73, p. 490.

Unfortunately there are many reasons, both theoretical and experimental, which show that such an explanation is inadmissible.

It was pointed out by Laqueur and Sackur* that the Na˙ ion in the case of the sodium salt of caseinogen is kept within the membrane of a dialyser by electrostatic forces alone. A similar state of affairs exists in the case of Congo red. The Na˙ ions tend to pass through the membrane in obedience to osmotic force. But they cannot travel beyond the distance at which the electrostatic attraction of the opposite ions, which are unable to pass the membrane, is equal and opposite to the osmotic pressure of the Na˙ ions. The osmotic pressure produced by the non-diffusible elements, the anions and the non-dissociated salt, shows itself in virtue of the mechanical constraint exerted by the membrane, which allows water to pass freely while holding back the bodies in question. In a similar way, the Na˙ ions are prevented from escaping owing to the constraint of the membrane on the opposite ions, so that the membrane must have to bear the pressure of both ions.† To put it in another way, the pull of the anions on the cations could not be effective unless the constraint of the membrane gave the former some *point d'appui*, so to speak.

We see, then, that what, at first sight, seems to be a reasonable view, viz. that the fact that the electrostatic forces balance the osmotic pressure of the diffusible ions prevents the manifestation of this pressure in an osmometer, will not stand examination.

Experimental evidence of various kinds, moreover, shows that all ions within the membrane are osmotically active. If direct measurements of osmotic pressure with membranes of parchment-paper did not give the full osmotic pressure of the whole of the bodies in solution inside the membrane, there would be a large discrepancy between values obtained in this way and those obtained in other ways—by vapour-pressure determinations, for example. The simple method of Barger‡ is sufficiently accurate for the present purpose. I found indeed, no difficulty in distinguishing between dilutions of cane-sugar of 45 and 50 litres. Two experiments were made with Congo red in dilutions of 30 and 50 litres. In the first case, it was found that the dye solution had a vapour pressure very slightly higher than that of saccharose in a dilution of 30 litres. The drops of dye solution at first took up a trace of water from the sugar solution and were then in equilibrium with it. Estimating from the change in dimensions of the drops, the dye solution had the same osmotic pressure as that of saccharose in a dilution of 29·4 litres. In the second experiment the drops of dye solution increased slightly in size when alternating with drops of saccharose at 50 litres dilution, but decreased when sugar at 45 litres dilution was substituted. The decrease in the latter case was greater than the increase in the former, so that we may take the actual osmotic pressure, or rather vapour pressure, of the dye solution to have been the same as that of sugar at a dilution of 48 litres. The results are sufficient to show that the direct measurements give correctly the total osmotic concentration. A very minute impurity, if of small molecular weight, would be capable of accounting for the very slightly higher vapour pressure of the dye solution than would be expected from the osmometer values; this, being diffusible, would not be shown by the direct method of estimation.

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* 'Beitr. z. chem. Phys. und Path. Hofmeister,' 1903, vol. 3, p. 203.

† See the forthcoming monograph on 'Colloids,' by W. B. Hardy, for a more detailed discussion of this question.

‡ 'Trans. Chem. Soc.,' 1904, vol. 86, p. 286.

If the $Na^{\cdot}$ ions were inactive osmotically, it is impossible that pressures higher than what I have called "molecular" could ever be obtained, even in solutions of great dilution. Now Biltz and v. Vegesack* themselves have obtained such. I have myself in dilutions of about 1000 litres seen on one or two occasions pressures of 101—102 per cent. of the "molecular"; but, since the actual values did not exceed 21 mm. Hg, I do not feel justified in drawing conclusions from them. On the other hand, with Chicago blue and with sodium caseinogenate pressures are always obtained higher than could be accounted for on the theory of Biltz.

Chicago blue is the tetrasodium salt of a substituted dinaphthylamine-tetrasulphonic acid.† If normally dissociated, it should give four sodium ions and one quadrivalent anion. If the former were inactive, the osmotic pressure of this dye should be the same as that of Congo red. In point of fact, a dilution of 353 litres gave a value of 93 mm. Hg or practically *double* the "molecular" osmotic pressure. It was the same, however, as that of a Congo-red solution of the same electrical conductivity. Another experiment, with a dilution of 1003 litres, gave an osmotic pressure of 35 mm. Hg, again double the "molecular" pressure.

The sodium salt of caseinogen, containing sufficient base to be faintly alkaline to phenolphthalein, gave an osmotic pressure of 313 mm. Hg for a solution of 3·23 per cent. If the anion alone were active, the maximum pressure could only be 155 mm. Hg, if it were quadrivalent only, and it may be higher in valency.*

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* 'Zeit. f. physik. Chem.,' 1910, vol. 73, p. 490.

† I am indebted to the kindness of the Berlin Aniline Company for a supply of this dye.

On the other hand, benzo-purpurin, which is of similar constitution to Congo red, but with tolidine in place of benzidine, gave me values of osmotic pressure only about 0·87 of the "molecular." The preparation used was that supplied by Kahlbaum, "recrystallised" by me five times. An analysis of its sodium content showed that it only contained 0·81 of what it should have contained if it had been the neutral (disodium) salt. It was probably a mixture of the mono- and di-sodium salts or of free acid and neutral salt.† Its osmotic pressure corresponded to that of a Congo-red solution of the same conductivity, as would be expected if the acid salt were in large colloidal aggregates and osmotically inactive, as was no doubt the case.

These sodium salts of non-diffusible organic acids appear to give osmotic pressures related to their sodium-content, although less than would be expected if they were dissociated normally.‡

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* See Laqueur and Sackur, 'Hofmeister's Beiträge z. Chem. Phys. und Path.,' 1903, vol. 3, p. 199. The fact that its osmotic pressure is double the "molecular," similar to that of Chicago blue, suggests that it is tetrabasic, like the acid of the dye.

† An interesting fact worth mention is that, if to a solution of Congo red there be added half that amount of a strong acid which is necessary for complete decomposition of the salt, instead of the acid salt being formed, as in the case of sodium sulphate, half the salt is decomposed with production of the free acid, while the other half remains in its original state of disodium salt. The solution becomes at first turbid and of a purple-brown colour. On standing, the blue free acid is deposited, leaving a clear solution of the bright red colour of the neutral salt. This behaviour appears to be due to the fact that the free acid is insoluble in water. If the whole be evaporated to dryness, the deposit extracted with methyl alcohol, in which the free acid is slightly soluble, the extract filtered, the filtrate evaporated again to dryness and taken up in water, a permanent colloidal solution of the acid salt is formed, turbid and of a purple-brown colour. It is difficult to say, however, whether this is a true salt or a colloidal complex, since it has to be remembered that it is in very dilute solution and contains no foreign electrolyte, facts which favour its stability.

As regards the free acid itself, it has been stated that the sodium salt may possibly not be decomposed even by strong acids, owing to the presence of the two sulphonic acid residues. I have recently tested this by precipitating the salt, by addition of sulphuric acid, and determining the sodium in the two phases. It was found to be entirely in the fluid phase as sulphate. The precipitate was merely the free acid, containing no salt.

‡ I have made one or two experiments recently with a dye known as "Primulin," which may be shortly described as the monosodium salt of a poly-thiazol-sulphanilic acid. It has a molecular weight of 608·5, and passes through parchment paper with extreme slowness. Its osmotic pressure appears to be between the "molecular" and half this value. In a dilution of 168 litres the pressure observed was 84 mm. Hg, the "molecular" being 118 mm. The sodium content of the preparation was, however, greater than corresponded to that of the salt of a monobasic acid. Its conductivity was that of a Congo-red solution giving an osmotic pressure of 140 mm. Hg. Further experiments with this dye are in progress.

Notwithstanding these facts, it is undoubtedly remarkable that the osmotic pressure of Congo-red solutions should correspond so closely to the "molecular" through a very wide range of concentration. Table II gives a number of experimental values corresponding to particular concentrations. In order to obtain those of the higher concentrations, it was found necessary to determine the difference between one of lower concentration outside the membrane and one of higher concentration within it. The osmotic pressure of the former was known from previous measurements.

Table II.

| Conductivity in recip. ohms × $10^6$. | | | Osmotic pressure in mm. Hg. |
|---|---|---|---|
| Inside. | Outside. | Difference. | |
| 6220 | 5070 | 1150 | 229 |
| 5545 | 5060 | 485 | 134 ·7 |
| 5033 | 4335 | 698 | 176 |
| 4360 | 3130 | 1230 | 223 |
| 3440 | 2470 | 970 | 172 |
| 2498 | 1762 | 736 | 122 |
| 1762 | 135 | 1627 | 245 |
| 1670 | 32 | 1638 | 263 ·5 |
| 1185 | 20 | 1165 | 178 |
| 705 | 18 | 687 | 108 |
| 409 | 22 | 387 | 33 (?) |
| 406 ·5 | 18 ·75 | 387 ·75 | 19 ·5 |
| 319 | 146 ·4 | 172 ·6 | 27 ·5 |
| 277 ·5 | 21 ·5 | 256 | 27 |
| 205 | 20 | 185 | 21 ·3 |

From these numbers a curve was constructed from which the osmotic pressure corresponding to any given conductivity could be obtained. From the previous conductivity measurements of solutions of known dilution, the dilutions corresponding to any given conductivity were known.

In Table III a series of such values are recorded.

Table III.

| Conductivity in recip. ohms × $10^6$. | Dilution in litres. | Osmotic pressure in mm. Hg. | "Molecular" osmotic pressure. | Percentage of "molecular" found. |
|---|---|---|---|---|
| 6772 | 14 | 1220 | 1328 | 90 |
| 5500 | 18 | 1020 | 1032 | 98 |
| 3782 | 28 | 625 | 664 | 94 ·5 |
| 2086 | 56 | 326 | 332 | 98 |
| 1154 | 112 | 176 | 166 | 106 |
| 662 | 224 | 106 | 83 | 127 |
| 367 | 448 | 45 | 41 ·5 | 108 |
| 201 | 896 | 24 | 20 ·8 | 114 |

All at 25° C.

In fig. 1 a curve is given correlating conductivity in recip. ohms × $10^6$ as abscissæ with osmotic pressure in mm. Hg as ordinates and in fig. 2 a curve with concentration in millimols. per litre as abscissæ and osmotic pressure in mm. Hg as ordinates.

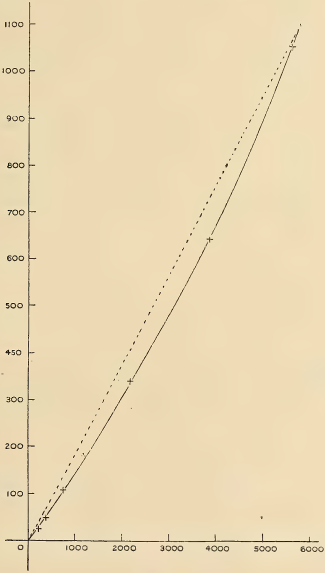

Fig. 1.

It will be noticed that the curve of fig. 1 is slightly convex to the axis of abscissæ, whereas that of fig. 2 is practically a straight line, although some of the points marked are somewhat divergent, as would not be unexpected in measurements of so much difficulty as direct determinations of osmotic pressure.

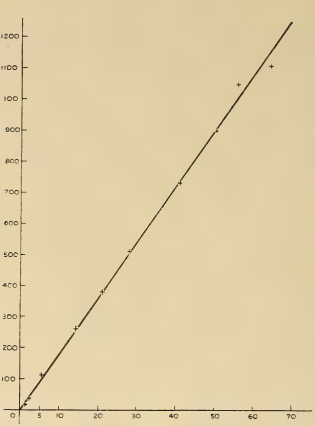

Fig. 2.

The apparent tendency of the numbers in the last column of Table III towards a maximum at 224 litres dilution is merely accidental, since other experiments do not show the phenomenon. Table IV gives some additional values.

Table IV.

| Dilution in litres. | Osmotic pressure obtained. | "Molecular" osmotic pressure. | Percentage of "molecular" found. |
|---|---|---|---|
| 15·5 | 1136 | 1200 | 94·8 |
| 17·8 | 1040 | 1044 | 99 |
| 19·55 | 902 | 950 | 95 |
| 24·2 | 726 | 768 | 94·7 |
| 35·55 | 508 | 523 | 97 |
| 47·2 | 386 | 394 | 98 |
| 73·5 | 253 | 251 | 101 |
| 110 | 178 | 169 | 105 |
| 203 | 107 | 92 | 111 |
| 445 | 40 | 42 | 96 |

The results of an application of v. d. Waals' formula to the present case have been worked out by Mr. Hardy, who informs me that, if such a formula applies, the osmotic pressure should be a linear function of the conductivity. Fig. 1 shows that this is not the case; the departure, however, although unquestionable, is not very great. The explanation may perhaps lie in the fact that the part played by the non-dissociated molecules in the production of osmotic pressure, and which would be relatively greater in the more concentrated solutions, is not taken into account. That these molecules do take part is shown by the fact that the increase of osmotic pressure for a given rise in conductivity shows a steady increase from the more dilute solutions upwards. For example, at 500 litres dilution, an increase of 50 recip. megohms in conductivity is associated with a rise in osmotic pressure of about 5·5 mm. Hg. At 20 litres dilution the same increase in conductivity gives a rise in osmotic pressure of 14 mm. Hg and there is a fairly regular transition from one to the other. At higher concentrations the rise per 50 recip. megohms falls to 10 again, but obvious precipitation commences to appear. The curve representing these ratios has an S-shape and is given in fig. 3, since it may turn out to be of some significance.

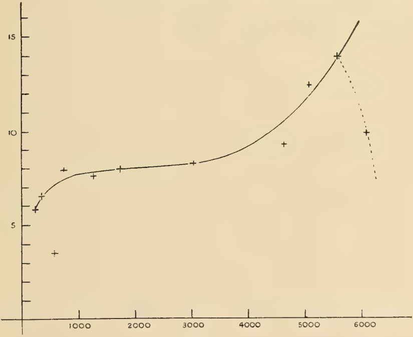

FIG. 3.

*Ordinates.*—Rise of osmotic pressure in mm. Hg for each 50 recip. megohms in conductivity.
*Abscissæ.*—Dilution in litres.

It may perhaps be possible ultimately to arrive at some knowledge of the relative parts played by the dissociated and non-dissociated molecules on the lines of these facts; the data at present available seem scarcely sufficient.

Why the osmotic pressure is a linear function of the molar concentration, although the dye is dissociated and all the ions are osmotically active, it is impossible to explain. It can only be suggested that the dissociation is not normal, but that complex ions are formed. Whether these are of the nature of chemical compounds, like the ferrocyanic ion, or whether they are associations similar to those of colloidal solutions, requires further investigation to decide.

### *Electrolysis of Congo Red.*

It was hoped that some light might be thrown on the nature of the ionisation of the dye by electrolysis of its solutions.

When the experiment is made in a U-tube containing the dye solution at the bend and above it, in both limbs, distilled water, and a current passed through platinum electrodes immersed in the water, the phenomena are complex and difficult to interpret. In the cathodic limb the boundary surface remains sharp, but a second sharp meniscus travels slowly towards the anode, so that behind it on the cathodic side the solution is pale red and in front of it deep red. The water on this side remains colourless. In the anodic limb there is also a sharp meniscus separating a lower deep red solution from a paler one above, but there is no definite boundary between this pale solution and the water above it. From the upper part of this indefinite boundary pale pink streamers pass through the water to the anode. When they reach this they become blue. I am inclined to regard these streamers as dissociated anions; when discharged at the anode the blue insoluble free acid is formed. I am unable to express an opinion as to whether the existence of a pale red instead of a colourless solution area travelling from the cathode means that $Na^{\cdot}$ ions were not present.

Other experiments were made by arranging three flasks in series, connected by glass syphon tubes and provided with platinum electrodes in the two end flasks. A current of about 2 milliampères was passed for five days, by which time it was calculated that sufficient current had passed to decompose the whole of the salt present. The amounts of dye-acid and of sodium were then estimated in the anodic and cathodic flasks, the sodium by the usual method of conversion to sulphate and the former by subtracting the sodium values from the total solids. It was found that the proportion of $Na^{\cdot}$ to anion in the positive flask was 1 to 1·9, and in the negative flask 4 to 1. If the proportion in the former had been 1 to 1, the dissociation equation might be

$$3\text{Ⓢ}Na_2 \rightleftarrows Na_2\text{Ⓢ}'' + \text{Ⓢ}Na_4^{\cdot\cdot},$$

where $\textcircled{S}$ stands for the non-diffusible anion. It is probable that there is an uncertainty about the concentration of the dye-acid in the positive flask owing to precipitation.

But this equation, even if otherwise acceptable, goes too far, since the osmotic pressure of the dissociated salt would be *less* than that of the non-dissociated. If it were

$$2\textcircled{S}\mathrm{Na}_2 \rightleftarrows \mathrm{Na}\textcircled{S}' + \textcircled{S}\mathrm{Na}_3^{\cdot},$$

the experimental facts would be satisfied as far as the osmotic pressure is concerned. There is difficulty, however, with the electrical conductivity. We have seen that the molar conductivity at infinite dilution is practically the same as that of sodium sulphate, if we allow for the slower migration rate of the large organic anion. Now one dissociated molecule of this salt carries four times as many charges as one of Congo red, according to the last equation, if sodium sulphate dissociates thus:—

$$\mathrm{Na}_2\mathrm{SO}_4 \rightleftarrows 2\mathrm{Na}^{\cdot} + \mathrm{SO}_4''.$$

Moreover, if we suppose that at infinite dilution Congo red is completely and normally dissociated, an osmotic pressure considerably exceeding the "molecular" as dilution increases would be given. This is so only to a very small degree, if at all, as we have seen. On the other hand, if the dissociation were of the type suggested, with complex ions, it seems impossible to explain the facts illustrated by the following case: at 28 litres dilution the molar conductivity of a Congo-red solution is half that at infinite dilution, whereas, on the supposition of complex ions, it would have only one-quarter the conductivity which it has at infinite dilution, even if it were completely dissociated at the higher concentration, a very unlikely assumption.

If, however, it were allowable to assume that the complex ions could retain the combined charges of their components, thus:—

$$2\textcircled{S}\mathrm{Na}_2 \rightleftarrows \mathrm{Na}^{\cdot}\textcircled{S}'' + \textcircled{S}''\mathrm{Na}_3^{\cdot\cdot\cdot},$$

the matter could be explained.

A similar difficulty was met with by Brailsford Robertson* in his investigation on the conductivity of the potassium salt of caseinogen. Under certain conditions the addition of potassium chloride to such salts causes no diminution of ionisation. The conclusion drawn is that potassium caseinogenate does not form $\mathrm{K}^{\cdot}$ ions. But, at the same time, it is pointed out that, in order to explain other experimental facts, it must be supposed that the complex ions are able to transport an increased number of atomic charges.

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* 'Journ. Phys. Chem.,' 1910, vol. 14, pp. 556 and 557.

There is one more hypothesis which might be made on the ground of the colloidal behaviour of the anions in the case of Congo red. These large organic ions may aggregate while retaining the combined charges of their components. This seems to imply, however, so great a density of the charge on the surface of the aggregates as to be improbable.* It could not, in any case, apply to the Na˙ ions, which cannot be assumed to form aggregates of this kind. If, however, we suppose that the non-dissociated molecules, as well as the anions, are so far aggregated as to give an osmotic pressure too small to be detected by a mercury manometer, so that the Na˙ ions alone are osmotically active, the osmotic pressure at a dilution such that the dissociation is 50 per cent. would be accounted for, but not when the dilution is greater than this, while the difficulty as to the large charge on the aggregated anions remains. The total charge of the anions must, of course, be equal and opposite to that on the cations.

The following experiment gives evidence, so far as it goes, against the formation of complex ions. When solutions of Congo red are exposed to electrolysis in a parchment-paper cup so that the anode is inside and the cathode outside, cations are driven out, as we have already seen. These ions are not coloured, as seems would be the case if they contained acid components. The outer fluid becomes strongly alkaline but remains colourless. It may be, perhaps, that under the action of a current the naturally present complex ions are caused to dissociate further, although the natural conclusion would be that the Na˙ ions were formed in the solution already.† It is of interest to note that, in this experiment, when the current is reversed, the escaped cations are driven back and that, after this has taken place, the current practically ceases to pass, owing to the inability of the anion to pass through the membrane. The apparatus is, in fact, a kind of rectifier.

On the whole it is evident that there is something abnormal in the form of the dissociation of salts, one of whose ions is colloidal. It may tentatively be suggested that aggregation of molecules may play some part in the mode of ionisation, so that this may be a function of the surface of the aggregates.

The fact that the observed values of the osmotic pressure are less than the "molecular" may be due to the presence of complex positive ions which are unable to pass the membrane. Thus, the undissociated molecules having only a low solubility, if complexes are formed, an aggregate consisting of an internal phase of particles, which ionise at the surface, *e.g.* :—

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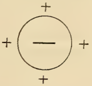
* It is pointed out to me by Mr. Hardy that the abnormally high velocity of the negative ion shows, either that resistance to movement decreases with increase of volume, or that the charge is increased. It might be argued that, as the normal velocity would be about $28 \times 10^{-5}$ cm. per second, the charge is 57/28 (*vide ante*), or twice as great as that carried by an ordinary ion.

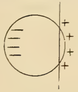
† This experiment cannot, perhaps, be regarded as offering very strong evidence against the presence of complex cations, since, if only a small fraction of the dye were dissociated with formation of Na˙ ions, these would pass through the membrane, and their place inside be filled by fresh dissociation, according to the law of mass action.

$$(\mathrm{Na_2}\textcircled{\scriptsize S})_n \rightleftarrows (\mathrm{Na_2}\textcircled{\scriptsize S})_{n-1}(\mathrm{Na}\textcircled{\scriptsize S})' + \mathrm{Na}^{\cdot},$$

might be produced owing to the solubility of the undissociated molecules being exceeded. In this way a typical condenser system is formed :—



At the membrane-face these particles would, by osmotic forces, be orientated thus :—



and so contribute to the electromotive force discussed in the following section.

Although Congo red is aggregated by foreign electrolytes there is some uncertainty as to whether there is any such association of its molecules in pure solutions. I stated in my previous paper on the osmotic pressure of Congo red that the ultra-microscope is unable to resolve such solutions. The path of the beam of light merely appears hazy. This haze may be the expression of aggregates beyond the limits of resolving power of the instrument used or it may be due to the individual molecules, or to anions, which must be of very considerable dimensions.

### *The Electromotive Force at the Membrane.*

If, in the dissociation of Congo-red solutions, complex or associated ions are produced, both anion and cation containing the organic acid grouping, it seems to follow that the membrane would be impermeable to both ions, so that no complication would arise as to the part played by the diffusible ion, since there is none.

But, whatever may be the precise nature of the ions in question, the following facts show that the cation is in point of fact diffusible and kept within the membrane by electrostatic constraint alone.

Lacqueur and Sackur pointed out* that, if the $\mathrm{Na}^{\cdot}$ ions in the case of sodium salts of caseinogen were kept within the membrane of an osmometer by electrostatic forces, this membrane itself would be the seat of a considerable potential difference.

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* 'Beitr. chem. Physiol. und Pathol. Hofmeister,' 1903, vol. 3, p. 203.

Hardy* has investigated the question mathematically from different points of view, (1) by consideration of the force acting upon the diffusible ions due to the potential difference between the two oppositely charged sides of the Helmholtz double layer as being equal and opposite to the osmotic pressure of these ions, and (2) by consideration of the work done in moving electricity from the lower potential to the higher. This latter is the method used by Nernst in the calculation of metallic electrode potentials and is based on Helmholtz' theory of contact potential. The same equation is arrived at by both methods:

$$\mathrm{E} = \mathrm{RT}/q \,.\, \log \mathrm{nat}\, c_2/c_1,$$

where E is the potential difference between the two sides of the membrane, R the gas constant, T absolute temperature, $q$ the charge on 1 grm. equivalent of the ion concerned, $c_2$ the concentration of this ion inside the membrane, and $c_1$ its concentration outside the membrane. Expressed in volts $\mathrm{RT}/q = 0{\cdot}0247$.

This equation, as will be noticed at once, has a striking similarity to the well-known one of Nernst expressing the potential at a metallic electrode, and may in a sense be regarded as an illustration of the way such a potential is produced. In any case it shows how a permanent electromotive force is produced by means of a membrane permeable to one ion only. It is quite different from that concerning the electromotive force of contact of two solutions of different concentration. Here the different rates of migration of the anions and cations are the cause of the potential difference, which only lasts until diffusion has put an end to concentration differences.

In attempting to apply this equation to actual experiment, I was struck by a result which follows from the above equation as also from that of Nernst on concentration batteries, but which seems to have escaped the notice of writers on the subject. If the outer fluid in the osmometer is distilled water, $c_1$, the denominator of the fraction, becomes zero, and the potential difference therefore infinite. The same applies to the concentration battery when the one solution is water. If the original paper of Nernst† be consulted, it will be found that this fact had not escaped his notice. He also points out that, theoretically, any diffusion into a vacuum should take place with infinite velocity; in ordinary cases this would only last for an infinitesimally short time. In actual practice, moreover, there is never an actual "vacuum" for electrolytic diffusion, since water itself is ionised and always contains impurities. For this reason, measurements are not reliable when $c_1$ is less than $m/1000$.

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* Forthcoming monograph on "Colloids."

† 'Zeits. f. physik. Chem.,' 1889, vol. 4, p. 139.

The side of the membrane in contact with the more dilute solution will be charged positively to the inner side, owing to the movement of the diffusible positive ion under osmotic forces in this direction until these forces are counterbalanced by electrostatic attraction of the negative ion.

In the experimental testing of the equation for the potential difference between the two sides of the membrane I have met with some difficulty. The first experiments were made with an osmometer of the pattern described by Roaf,* constructed of glass and ebonite only. A T-tube in the course of that leading to the manometer was connected with a tube containing Congo-red solution of the higher concentration, as contained in the osmometer, but made stiff with gelatin in order to withstand the pressure. In later experiments, it was found unnecessary to use the gelatin. The osmometer was immersed in the more dilute solution, from which a syphon tube dipped into Congo-red solution of the same concentration as that within the osmometer. Into this solution the tube of an Ostwald calomel electrode was immersed, while a second similar electrode was in connection with the content of the osmometer, with or without the gelatin protection. The system was, therefore, symmetrical, with the exception of the presence of the parchment-paper membrane between the two solutions of the dye on the one side. The electromotive force was measured by compensation with a potentiometer in the usual way, using a capillary electrometer as indicator.

It was found that the sign of the potential difference was as deduced from theoretical considerations, viz., the side of the membrane in contact with the more dilute solution was positive to the other side, or, as the stronger solution was always inside the osmometer, the outside was positive to the inside. But values corresponding to those calculated from the equation given above were obtained, as a rule, only immediately after the apparatus was put together. In one such case, solutions of 30 and 80 litres dilution gave an electromotive force of 0·025 volt against the calculated one of 0·022 volt. This value soon fell to 0·0136 volt, although the manometer had not perceptibly fallen. On the next day, however, it had risen again to 0·0165 volt.

In another experiment, in which the relative ionic concentration of the two solutions was determined, by conductivity measurements, to be $m/32$ and $m/150$ respectively, the E.M.F. found was 0·025 volt, instead of 0·022 volt calculated. With more dilute outer solution, the E.M.F. observed was 0·068 volt instead of 0·088 calculated, but in this case the concentration of the outer solution was too low for accurate measurements, and the reading was not made until some time after setting up.

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* 'Quart. Journ. Exp. Physiol.,' 1910, vol. 3, p. 80.

The reason why the E.M.F. steadily falls for a time after setting up the apparatus is, I think, to be explained by the fact that it is almost impossible to prevent a small diffusion of water through the membrane, owing to the high osmotic pressure within. The difference of concentration must be considerable in order to obtain sufficiently high potential differences for accurate measurement. The dilute layer next the membrane will only slowly diffuse into the rest of the solution and as long as it is contact with the membrane, the conditions are not those corresponding to the concentration of the main bulk. It seems then that the values obtained before the water has had time to pass through are the more reliable. When the osmometer was replaced by an open parchment-paper dish, the E.M.F. fell so rapidly that it was impossible to obtain satisfactory readings. In a special osmometer constructed by cementing parchment-paper over the end of a tube of 8 mm. diameter, it was found that, with solutions of conductivities of $3950 \times 10^{-6}$ and $1955 \times 10^{-6}$ recip. ohms respectively, an E.M.F. of 0·0149 volt was found, with an osmotic pressure of 288 mm. Hg at 21° C. The calculated value was 0·0174 volt, so that 86 per cent. of the theoretical number was obtained.

It occurred to me that, perhaps, by sending a continuous current of the more concentrated solution through the interior of a parchment-paper tube and one of the dilute solution over the outside, more permanent results might be obtained. The figures were:—

| | |
|---|---|
| Conductivity of the stronger solution ......... | $5780 \times 10^{-6}$ recip. ohms. |
| „ dilute „ ......... | $192 \times 10^{-6}$ „ |

E.M.F. calculated, 0·083 volt; found, 0·07 volt, or 84 per cent. of theory.

Since, in these experiments, the osmotic pressure is the sum of that of the ionised and non-ionised salt, while the E.M.F. is due only to the former, it should be possible, by calculation of the osmotic pressure corresponding to the potential difference as measured and subtracting this from the observed osmotic pressure, to determine the relative proportion of the ionised and non-ionised parts of the salt in the osmometer.

It is worth recording that the E.M.F. can be at once abolished by replacing the outer solution by one of sodium chloride of the same ionic concentration, as estimated by electrical conductivity, as the solution on the other side of the membrane. If the solution thus placed outside is more concentrated in $Na^{\cdot}$ ion than that inside, the sign of the potential difference is reversed, as would be expected. This reversal lasted longer than would be supposed from the fact of the diffusibility of the sodium chloride, viz., 18 hours at least. If the outer solution be replaced by water while the sign is reversed, this latter is changed back again to the original direction, but is naturally greater in numerical value owing to the previous diffusion of sodium chloride into the inner solution, which gradually escapes again into the water.

The fact that the E.M.F. given by Congo-red solutions of unequal concentration separated by a membrane can be abolished or reversed by $Na^{\cdot}$ ions does not, it seems to me, warrant the conclusion that the dye solutions necessarily dissociate with the production of $Na^{\cdot}$ ions. If I rightly understand the rationale of the process, the production of the potential difference under discussion is a matter of electric charge of positive sign, so that the precise chemical nature of the carrier of the charge is a matter of indifference. That this point of view is correct is shown by the fact that potassium chloride, as well as sodium chloride, is effective in reducing the E.M.F. A Congo-red solution in dilution of about 21 litres gave, with water outside the membrane, an E.M.F. of 0·05 volt; when the water was replaced by $m/20$ potassium chloride, this value was reduced to less than 0·001 volt. This being the case, $c_1$ and $c_2$ in the equation must apparently be regarded as expressing the concentrations of positive charges. The abolition of E.M.F. is, of course, only a temporary one, since the $K^{\cdot}$ ions diffuse through the membrane and thus produce again a concentration of positive ions greater on the inner side of the membrane. A potential difference due to the greater migration rate of the potassium ion than that of the sodium ion would not, I think, be perceptible under the conditions of the experiment. It is of interest to note that this experiment shows how sodium ions are enabled to escape by interchange with similarly charged ions in the outer fluid.

The possibility that the membrane itself may carry an electric charge must not be neglected, although the concordance of the most reliable of the experimental results given above with the calculated values shows that, if so charged, this charge plays no appreciable part in the production of the E.M.F. Moreover, it would only be very small in the presence of the large electrolyte concentration on both sides. As far as osmotic pressure is concerned, *à priori* considerations, for which I must refer to Mr. Hardy's forthcoming work on "Colloids," show that a charge on a membrane could only affect the time taken to attain equilibrium and would have no effect on the permanent result, unless the permeability of the membrane were affected. The total constraint exerted by the membrane, upon the non-dissociated molecules and anions directly, upon the cations indirectly, would be unaffected.

Since the cation is held within the membrane by electric forces alone, it is to be expected that, by passing an electric current through the membrane by putting a platinum anode inside the osmometer and a similar cathode in the outer solution, the cations would be enabled to escape. This does in fact happen. The osmotic pressure falls, the solution inside becomes turbid owing to excess of free acid, while the outer fluid becomes alkaline. If the current be stopped and the outer alkaline solution be replaced by water, the osmotic pressure does not rise again until free alkali is added to replace that which has been allowed to diffuse out by the agency of the electric current. If the cation were a complex one containing colour acid it might be supposed that the outer fluid would be coloured in the above experiment. This, in point of fact, was the case to a slight degree. But parchment-paper is not absolutely impermeable to Congo red and the permeability is somewhat increased by the presence of alkali. The depth of colour did not seem to be greater than would have been attained in the absence of the electric current, and in the experiment described in the previous section, made for another purpose, no colour passed through the membrane during the time of the experiment, although considerable amounts of sodium hydroxide were formed.

The degree to which parchment-paper of the thick kind used in my experiments is permeable to the dye is actually very small. In an experiment in which the outer water in the osmometer was not changed for ten weeks the dye contained therein only amounted to 0·0078 per cent.

There is a slight possibility that, in a metallic osmometer, closure of a circuit between the two sides of the membrane may, to a very small extent, enable $Na^{\cdot}$ ions to escape. It seems most probable that polarisation would rapidly annul such a current. As already stated, moreover, no diffusion of alkali can be detected in the absence of an extraneous E.M.F.

The problem dealt with in this section is closely connected with a fundamental difficulty in the Arrhenius theory of electrolytic dissociation. According to the kinetic theory, the dissociated ions with which we are concerned are free from mutual influence upon one another, so that there should be no electrostatic attraction to prevent the diffusible ions from passing freely through the membrane. The origin of the energy required to separate the ions is a matter of some uncertainty. It has been suggested by Larmor* that "internal potential energy is released owing to the ions entering into relations of closer affinity with the solvent," and by Bousfield and Lowry† that the affinity of the "ionic nucleus" for water is the main source of the energy required.

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* "Wilde Lectures," 'Mem. Manchester Lit. and Phil. Soc.,' 1908, vol. 52, Mem. 10, p. 37.

† 'Trans. Faraday Soc.,' 1907, vol. 3, p. 125.

### *The Distribution of Salts between Congo-red Solutions and Water separated by a Membrane.*

The electrical conditions within the membrane are undoubtedly the cause of the peculiar distribution of a foreign salt between the dye inside and the water outside.

The salt used in my experiments was chiefly sodium chloride, on account of the ease with which chlorine can be estimated by Volhard's method. In order to do this in the presence of the dye, it was found sufficient to precipitate the dye acid in a known volume by addition of excess of nitric acid and filtering off the precipitate before the actual Volhard determination. Tests were made in order to see whether any chloride was carried down with the precipitate of the dye acid. This was found not to be the case.

When sodium chloride is added to either the inner or outer solution in an osmometer there is a considerable fall in osmotic pressure, if sufficient time be allowed for equilibrium to be established. This fact was described in my former paper. If the chlorine content of the two solutions, inside and outside the membrane, be estimated at this time, it is invariably found that the outer fluid, from which dye is absent, contains a *larger* percentage of chloride than the inner dye solution. The difference is more marked the greater the concentration of the dye salt in relation to that of the sodium chloride. Table V gives a few of the measurements made. The numbers are dilutions in litres.

Table V.

| Dye. | Chlorine. | |
|---|---|---|
| | Inside. | Outside. |
| 30 | 52 | 30 |
| 30 | 465 | 73·6 |
| 30 | <5500 | 180 |
| 100 | 32·9 | 29·5 |

I regret that my experiments are not sufficiently numerous to enable a complete theory to be given of this partition, but there are two views which must be referred to.

Donnan* has investigated the question from the thermodynamic point of view, and comes to the conclusion that, as far as the non-dissociated salt is concerned, there must be equal distribution between the two sides of the membrane. This seems quite what would be expected, since there are no forces to prevent free diffusion of bodies devoid of electric charge. Moreover, the proportion of non-dissociated to dissociated salt is naturally *greater* within the membrane, owing to the diminished ionisation in the presence of the dye which has an ion ($Na^{\cdot}$) in common with the sodium chloride. This proportion will also be greater the greater the concentration of the dye. The result will be that the total concentration of sodium chloride will be less within the membrane, since less will be necessary in order to provide the same amount of non-dissociated salt.

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* In the Press. Prelim. Communication to the Physiological Society, December, 1910.

On the other hand, Biltz and v. Vegesack* maintain that the concentration of diffusible ions becomes equal on both sides of the membrane, diffusible ions including the cations of the dye. Consideration of the case where the dye solution is of high concentration and the sodium chloride dilute shows that this view does not afford a sufficient explanation. Such a case is the third one in Table V. The chlorine in the inner solution was, in fact, too small to be accurately estimated; a faint turbidity only was produced by the addition of silver nitrate. The diffusible ions of the inner solution would have a concentration of at least $m/30$, since at the concentration in question the dye is more than 50 per cent. dissociated. The ions of the sodium chloride outside could not have a concentration greater than $m/90$ if the salt were completely dissociated. The investigators referred to find that the conductivity is equal on both sides of the membrane, as their theory requires, when a relatively large concentration of sodium sulphate is present along with a dilute solution, 0·0007325 molar, of benzo-purpurin within the membrane. I have always found that the conductivity of the inner solution containing the dye is greater than that of the outer one, although, of course, when the proportion of foreign electrolyte is very great, the difference comes very near the limit of detection.

The actual values of the conductivity of the inner solutions found in my experiments afford, as far as they go, confirmation of the theory put forward by Donnan. For example, in the first experiment of Table V, the conductivity of the inner solution was found to be 5920 recip. megohms. That of the dye alone could not be greater than 3935 recip. megohms, so that the ionised sodium chloride must have had at least a conductivity of 1985 recip. megohms, which is that of an $m/60$ solution at the temperature of the experiment. The amount of chlorine found corresponded to $m/52$ NaCl, so that $(m/52 - m/60 =) \, m/400$ was present non-ionised. It has been assumed, however, that there was no depression of the ionisation of the dye by the sodium chloride. If there were such an effect, the concentration of the ionised sodium chloride would be greater than $m/60$, and therefore that of the non-ionised less than $m/400$. The outer solution, if 90 per cent. dissociated, would have a concentration in non-dissociated salt of 10 per cent. of $m/30$, or $m/300$. If its dissociation had been 87 per cent., the concentration of the non-ionised salt would have been $m/400$. The result may then be taken as supporting Donnan's theory.

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* 'Zeits. f. physik. Chem.,' 1910, vol. 73, p. 492.

The marked effect of foreign electrolytes in depressing the osmotic pressure of Congo-red solutions described in my former paper is, for the most part, due to this peculiar distribution of the salt between the two sides of the membrane. In the third experiment of Table V the osmotic pressure actually observed was 400 mm. Hg. That of the dye against water should have been about 620 mm., that is 220 mm. higher than that found. But the osmotic pressure of the $m/180$ sodium chloride in the outer fluid would be 206 mm., which must be deducted from the 620 mm., leaving 414 mm. instead of 400 mm. observed. The osmotic pressure of the small amount of the sodium chloride within the membrane, being less than 3 mm. Hg, may be neglected. The difference between the observed and calculated values is probably to be accounted for by aggregation of the dye induced by the foreign salt, which would not be great, since the amount of this salt within the membrane was so small.

### *The Action of Carbon Dioxide.*

It remains to refer to the effect of the presence of carbon dioxide or other acid in the outer fluid of the osmometer.

Various observers have noticed the difficulty in obtaining anything like permanent pressures with colloidal salts, such as those of caseinogen, similar to those dealt with in the present paper. This fact is due to the access of carbon dioxide in the air.

In dealing with colloidal systems, which are essentially unstable, it is not to be expected that really permanent osmotic pressures are to be obtained. If the manometer readings in my experiments did not fall more than by 2 or 3 per cent. of their values in 24 hours I considered it justifiable to assume that equilibrium was established between the two sides of the membrane. Experiments showed that equilibrium between diffusible bodies took place in the form of osmometer used in less than 24 hours. In the experiments of Biltz and v. Vegesack* on benzo-purpurin the pressure fell from 9·6 to 5 cm. of solution in 38 hours and to 0·73 in 84 hours. That it is possible to obtain practically constant pressures for several days, if carbon dioxide is effectively excluded, is shown by the following manometer readings from one of my experiments with benzo-purpurin, "conductivity" water being outside and frequently changed before these readings were taken :—

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* 'Zeits. f. physik. Chem.,' 1909, vol. 68, p. 367.

| Date. | | Date. | |
|---|---|---|---|
| 1910. | | 1910. | |
| May 25 ......... | 49 | May 31 ......... | 49 |
| „ 27 ......... | 49 | June 3 ......... | 48 |
| „ 30 ......... | 48·5 | „ 6 ......... | 48 |
| | In all 288 hours. | | |

The effect of carbon dioxide was described in my former paper. In order to discover how this effect is produced I have made further experiments as follows :—

A solution of Congo red was placed in an osmometer of Graham's pattern and carbon dioxide passed for some time through the outer water. It was found that the dye became purple-brown in colour and for the most part precipitated. The outer water on evaporation to dryness deposited crystals of sodium carbonate. The explanation of this fact is clearly that when $H^{\cdot}$ ions are present in the outer fluid there is no hindrance to the free interchange between these and $Na^{\cdot}$ ions through the membrane. Accordingly the latter pass out and combine with $CO_3'$ ions when the solution is concentrated by evaporation. The hydrogen ions entering the dye solution cause aggregation of the acid salt formed.

Graham* noticed that if he placed within a dialyser a solution of the sodium salt of albumen, the whole of the sodium was ultimately found in the outer water in combination with carbon dioxide derived from the air. I find that a similar loss of sodium occurs with the sodium salt of caseinogen.

Biltz and v. Vegesack† state that solutions of Congo red through which carbon dioxide has been passed recover their original state when boiled, even supposing that they have been dialysed. I find that this statement is correct for non-dialysed solutions ; as the carbon dioxide is driven off, the dye acid displaces more of it from the sodium carbonate and enters itself into combination with the sodium. If the solution has been dialysed after subjection to the action of carbon dioxide, so that the sodium carbonate has been removed, I find it impossible to regenerate the dye by boiling, provided that this be done in a quartz flask fitted with a reflux condenser also of quartz. The corresponding experiment of Biltz and v. Vegesack‡ was made with a

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[*] 'Phil. Trans.,' 1861, vol. 151, p. 217.

[†] 'Zeits. f. physik. Chem.,' 1910, vol. 73, p. 487.

[‡] 'Zeits. f. physik. Chem.,' 1910, vol. 73, p. 488.

very dilute solution of the dye, and required boiling for two hours with a reflux condenser, presumably of glass, in order to regenerate it. I think it quite possible that sufficient alkali was dissolved from the glass to account for the result.

There is, also, apart from the action of carbon dioxide, a slow aggregation in solutions of Congo red, and especially of benzo-purpurin. This change is reversed by heating. For example, a dilution of the latter dye of 94 litres, which had remained for some time in the osmometer with frequent changes of water free from carbon dioxide, had an osmotic pressure of only 68 mm. Hg, or 35 per cent. of the "molecular." After heating to 86° C. the osmotic pressure rose to 175 mm. Hg, or 88 per cent. of the "molecular."

### *The Temperature Coefficient of the Osmotic Pressure of Congo Red.*

Some incidental observations on the effect of temperature seem to show, as far as they go, that the osmotic pressure of this dye is proportional to the absolute temperature. A solution which had an osmotic pressure of 123 mm. Hg at 28·5° C. showed one of 138 mm. Hg at 62° C. Now

$$123 \times \frac{273+62}{273+28{\cdot}5} = 137.$$

### *Summary of Conclusions.*

1. No hydrolytic dissociation is to be detected in solutions of Congo red, and merely a trace, if any, in sodium caseinogenate.

2. On the other hand, electrolytic dissociation occurs to a large degree. Carefully purified Congo red in dilutions of 28 litres is 50 per cent. ionised; in 500 litres, 80 per cent. Although considerable, however, it is not so great as that of sodium salts of other organic acids of small molecular weight at corresponding dilutions, probably owing to colloidal aggregation in the case of the solutions of the dye salt.

3. The osmotic pressure found experimentally, both by direct measurement and by vapour pressure, is, throughout a wide range of concentration, uniformly between 95 and 100 per cent. of what it would be if no dissociation existed. Since it should be from one and a half to three times this value, according to the concentration, it is plain that there are some abnormal conditions present.

4. The sodium ion being kept within the membrane merely by electrostatic forces, it might be supposed that it is inactive in the production of osmotic pressure. The agreement of vapour pressure with direct determinations is sufficient to show that this is not the case. Moreover, Chicago blue, consisting of a single large non-diffusible anion, like Congo red, but with four

$Na^{\cdot}$ ions instead of two, gives *double* the osmotic pressure of the latter at the same concentration, whereas it should be the same, on the view of the non-activity of the diffusible ion. The suggestion is also disproved by theoretical considerations.

5. The curve expressing the ratio of the conductivity of Congo-red solutions to their osmotic pressure is convex to the axis of abscissæ when these are the values of the conductivity. It is a straight line when expressing the relation of osmotic pressure to molar concentration.

6. The value of osmotic pressure per unit increase of conductivity rises with concentration, forming an **S**-shaped curve.

7. Difficulties are pointed out in the hypothesis of formation of complex ions if these are supposed to contain both acid and base components. The possibility of aggregated simple ions carrying the sum of the charges of their components is suggested in order to explain the experimental results.

8. Whatever may be the nature of the cation, that it is diffusible is shown by the fact that the membrane is the seat of an electromotive force. The sign and numerical values of this potential difference agree, within experimental errors, with the equation deduced by Hardy.

9. The distribution of a foreign salt, such as sodium chloride, between the solution of the dye on one side of the membrane and water on the other side is always such that its concentration is greater in the water. Numerically the results agree with Donnan's view of equality of concentration of non-ionised sodium chloride on both sides of the membrane, and not with that of Biltz and v. Vegesack of equality of total diffusible ions. The effect of sodium chloride on the osmotic pressure is chiefly due to this peculiarity of distribution, since its aggregating effect is relatively small.

10. It is impossible to obtain even an approximately constant osmotic pressure in the case of colloidal salts with a diffusible cation if carbon dioxide be allowed access to the outer water of the osmometer. This effect is shown to be due to the fact that the presence of $H^{\cdot}$ ions in the outer fluid allows $Na^{\cdot}$ to escape by interchange of ions. The final result is the escape of the greater part of the sodium from the interior and precipitation of the acid salt.

11. Congo red appears to obey the gas law so far as the effect of temperature on the osmotic pressure of its solutions is concerned.

The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society.

# On the Fate of Red Blood Corpuscles when Injected into the Circulation of an Animal of the same Species; with a New Method for the Determination of the Total Volume of the Blood.

By Charles Todd, M.D., Bacteriologist, Egyptian Government; and
R. G. White, M.B., Director, Serum Institute, Cairo.

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(From the Hygienic Institute, Public Health Department, Cairo.)

In a paper published last year* the authors described a method by which it is possible to recognise the red blood corpuscles of any individual ox and to differentiate them from those of any other member of the same species.

The method depends upon the fact that if a highly polyvalent isohæmolytic serum is treated repeatedly with the red blood corpuscles of any individual of the species for which the serum has been made, it entirely loses its hæmolytic action for the corpuscles of all other individuals of the same species.† Such a serum therefore constitutes, so to speak, a specific reagent for the corpuscles of the individual for which it has been prepared, and by its means one is enabled to follow up and to identify these corpuscles even in the presence of corpuscles of other individuals.

Being in the possession of such a method we were led to investigate the fate of the red blood corpuscles of one animal when these are injected into the circulation of another animal of the same species, as in ordinary transfusion. As the serum at our disposal was prepared for cattle, the investigations were made with the blood of these animals, and the experiments were carried out as follows:—

Two suitable bulls (A and B) having been chosen, each was bled from the jugular vein—about 100 c.c.—in order to obtain the required corpuscles, which were washed in normal saline, four washings being found sufficient for this purpose.

A highly polyvalent isohæmolytic cattle serum was then "exhausted" for the corpuscles of A by being mixed with an equal volume of these corpuscles. The mixture was allowed to stand for an hour at 37° C. and then centrifuged—the supernatant serum being pipetted off and again treated with an equal volume of the same corpuscles, and the process repeated four times. The serum so treated was now found to be completely "exhausted" for the corpuscles of A, that is to say, in the presence of fresh guinea-pig serum it showed no trace of hæmolysis with the corpuscles of A, but remained powerfully hæmolytic for those of B.

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* 'Roy. Soc. Proc.,' 1910, B, vol. 82.

† This rule is liable to certain exceptions in the case of close blood relations.

Another lot of serum was similarly treated with the corpuscles of B, and in this way a serum was obtained which was "exhausted" for the corpuscles of B but remained powerfully hæmolytic for those of A.

The two exhausted sera so prepared now constituted specific reagents for the corpuscles in question, and by means of these reagents it was possible to analyse a mixture of the two corpuscles by dissolving out at will either of the corpuscles with the corresponding serum.

The exhausted sera having been prepared and tested, the animals were each bled from the jugular vein into graduated vessels containing a known volume of a 4-per-cent. solution of sodium citrate, from 2 to 4 litres of blood being taken from each animal. The citrated blood from each animal was then transfused into the jugular vein of the other, the whole process being carried out as quickly as possible and the blood being kept at body temperature.

After an interval of about a quarter of an hour, to ensure a uniform distribution of the injected blood in the circulation, a sample was taken from each animal and allowed to run into citrate to prevent clotting. Similar samples were taken daily throughout the experiment.

The examination of these samples was carried out thus:

A 2·5-per-cent. suspension of the sample blood was made in normal saline. This was centrifuged to get rid of serum and the corpuscles resuspended in saline. This suspension was then tested by being mixed with equal volumes of the exhausted serum and a 1/10 dilution of fresh guinea-pig serum.

The test put up as follows:—

1. 2·5-per-cent. suspension of red blood corpuscles ...
   Serum exhausted with corpuscles of Bull A ......
   1/10 dilution of fresh guinea-pig serum ............ } 0·5 c.c. of each.

In this tube the corpuscles of B are picked out and hæmolysed: those of A remaining unchanged.

2. 2·5-per-cent. suspension of red blood corpuscles ...
   Serum exhausted with corpuscles of Bull B ......
   1/10 dilution of fresh guinea-pig serum ............ } 0·5 c.c. of each.

Here the corpuscles of A are dissolved, those of B remaining unchanged.

3. 2·5-per-cent. suspension of red blood corpuscles ... 0·5 c.c.
   Normal saline ............................................ 1·0 „

Here both corpuscles are present, giving the total.

The tubes are kept in the incubator at 37° C., being thoroughly shaken from time to time, and at the expiration of two hours the red blood corpuscles are counted by the Thoma-Zeiss apparatus.

Eight animals were injected in this way with quantities varying from about 2 to 4 litres, and in all these animals a similar course of events was observed, the number of the foreign corpuscles in the circulation gradually diminishing until they disappeared; the disappearance took place after a lapse of from four to seven days after the injection and appeared to be related to the amount of blood injected. Shortly after the disappearance of the foreign corpuscles from the circulation, the blood serum began to acquire hæmolytic properties.

The annexed curve showing the results of the blood counts in one particular animal (Bull No. 72) may be taken as typical of the general results. This animal, a Cyprus bull weighing 412 kilos., was bled 2½ litres from the jugular vein. It then received an intravenous injection of 2540 c.c. of the freshly drawn and citrated blood of another bull (No. 73); this volume of citrated blood corresponds to 2117 c.c. of pure blood. About a quarter of an hour later a sample of blood was taken from the jugular vein and run into citrate, and similar samples were taken every day.

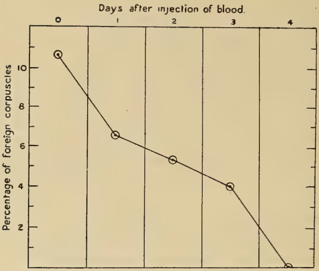
On examination by the method previously indicated the following results were obtained:—

Bull No. 72.

| Date. | No. of red blood corpuscles per square of Thoma-Zeiss apparatus. | | Percentage of foreign corpuscles. |
|---|---|---|---|
| | Foreign. | Own. | |
| 1911. | | | |
| April 30 ...... | 1·4 | 11·7 | 10·7 |
| May 1 ...... | 0·77 | 10·7 | 6·7 |
| " 2 ...... | 0·58 | 10·3 | 5·3 |
| " 3 ...... | 0·44 | 10·6 | 4·0 |
| " 4 ...... | 0 | | 0 |

The above results are shown as ordinates on the annexed curve.

It will be seen from this curve that immediately after the transfusion the foreign corpuscles represented 10·7 per cent. of the total number of corpuscles in the blood, and that this percentage steadily decreased until the expiration of four days, when they had completely disappeared. The disappearance of the foreign corpuscles was followed by the gradual



appearance of a hæmolysin for the corpuscles of Bull No. 73 as shown in the following test:—

*Testing Hæmolytic Power of Serum.* Bull No. 72.

Blood transfused on April 30, 1911.

Foreign red blood corpuscles disappeared on May 4, 1911.

Test put up as usual with—

1. Complement 1/10, 0·5 c.c.
2. Serum, varying amounts.
3. Red blood corpuscles of 73 (5-per-cent. washed), 0·5 c.c.

Serum of 72.

| Date. | 0·5 c.c. | 0·2 c.c. | 0·1 c.c. | 0·05 c.c. | 0·02 c.c. |
|---|---|---|---|---|---|
| 1911. | | | | | |
| May 5 | Trace | Nil | Nil | Nil | Nil |
| „ 6 | Almost C. | Marked H. | Trace | Nil | Nil |
| „ 7 | C. | C. | C. | Marked H. | Nil |
| „ 8 | C. | C. | C. | Marked H. | Nil |
| „ 9 | C. | C. | C. | Almost C. | Nil |
| „ 11 | C. | Almost C. | Trace | Nil | Nil |
| „ 12 | C. | C. | C. | Almost C. | Nil |

C = Complete hæmolysis. H = Hæmolysis.

The above figures for the red blood corpuscles are approximately correct, but no great degree of accuracy was aimed at. The question of greater accuracy merely involves the counting of a larger number of squares in the Thoma-Zeiss instrument, which was not considered necessary in these experiments.

The accuracy of these determinations can obviously be controlled by an estimation of the amount of hæmoglobin liberated, and the employment of a suitable hæmoglobinometer, which we unfortunately did not have at our disposal, might save the laborious counts involved in a Thoma-Zeiss examination.

The results of these experiments emphasise in a most striking manner the marked individuality of the red blood corpuscles, and we see that the injected corpuscles are not merely not accepted by their host, but are regarded as definitely foreign, and in fact functionate as antigens, and give rise to the formation of corresponding antibodies in accordance with the general laws of immunity. The bearing of these facts on transfusion as practised in medicine is obvious.

Another interesting point in these transfusion experiments is that they give us the necessary data for the determination of the total mass of the blood in the transfused animals, and this method would appear to have the advantage of a very considerable degree of exactitude, as the latter is only limited by the errors of the Thoma-Zeiss apparatus. As this was not the purpose of our experiments, in most cases the amount of blood injected into the animals was not accurately measured, but three bulls which did receive accurately measured volumes of blood gave results showing the total mass of the blood to be 1/17·3, 1/17·0, 1/18·7 respectively of the total body weight, assuming the sp. gr. of the blood to be 1050.

The animal experiments in the above work were made at the Government Serum Institute at Abbassia, and we are indebted to Mr. Gordon, Veterinary Surgeon to the Institute, for much valuable help in carrying them out.

### *Conclusions.*

1. The employment of specifically exhausted isohæmolytic sera affords a method of quantitatively analysing mixtures of the red blood corpuscles of different individuals of the same species.

2. By means of this method it is possible to follow out the red blood corpuscles of one individual when these are injected into the circulation of another animal of the same species.

3. When examined by these methods it is found that the injected corpuscles are treated by their host as foreign, and in fact act as antigens, and give rise to the formation of corresponding antibodies in accordance with the ordinary laws of immunity.

4. Transfusion experiments investigated by this method gave relatively accurate data for the estimation of the total mass of the blood.

# *Electrical Effects accompanying the Decomposition of Organic Compounds.*

By M. C. Potter, Sc.D., M.A., Professor of Botany in the University of Durham.

(Communicated by Dr. A. D. Waller, F.R.S. Received July 14, 1911.)

The results of recent researches in electro-physiology have familiarised us with the view that any physiological process accompanied by chemical changes involves an associated electrical change. Haacke and Klein have shown that electrical currents in plants are essentially a manifestation of vital phenomena, and that differences in electric potential are connected both with respiration and carbon assimilation. Waller's investigations have also shown that the excitation of living vegetable protoplasm gives electrical response no less than that of animal protoplasm. He has demonstrated that leaves in a condition of active metabolism give an instant electrical response to the influence of sunlight, which was modified under conditions affecting protoplasmic activity. Apparently almost immediately upon the perception of the stimulus of light, electrical energy begins to be absorbed in the process of photosynthesis. Waller approaches very suggestively the existence of two opposing forces in the presence of analytic and synthetic processes, and recognises that the functions of assimilation and respiration might be mutually antagonistic as regards visible electric effects. His conception that "the product of dissociation . . . . gives current *from* the focus of dissociation, whereas a product of association, during its formation, gives rise to a current in the opposite direction," is of great interest.

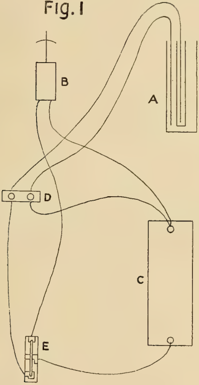
The line of enquiry now followed lies in the direction only of dissociation, and is a study of electrical effects accompanying fermentation or putrefaction under the influence of micro-organisms such as *Saccharomyces* or bacteria. The special physiological character of fungi or bacteria demands the disintegration of organic compounds as a necessary source of energy, and where there has been absorption of energy in a synthetic process one must look for its liberation when the change is of an analytic nature. The evolution of caloric energy during fermentation or putrefaction is commonly recognised, and that electrical energy is also liberated during these processes is a conception of considerable interest. In this preliminary communication some experiments are described which were undertaken to determine whether any E.M.F. is developed when organic compounds are broken down through the fermentative activity of yeast and other organisms. Cultures of

*Saccharomyces cerevisiæ* and certain species of bacteria were grown in nutrient media, and the chemical action of their vital processes was utilised to develop electrical energy in a manner parallel to the production of E.M.F. by means of the ordinary galvanic cell.

### *The Apparatus.*

The apparatus employed (fig. 1) consisted of a glass jar containing a porous cylinder. The same nutrient fluid was placed in the glass jar and in the porous cylinder, and two platinum electrodes were introduced, one into the fluid in the jar and the other into the porous cylinder. In the case of pure cultures, instead of the jar, a large boiling tube was used, which could be plugged with cotton wool and the whole apparatus sterilised either by intermittent boiling or in an autoclave. After sterilisation the fluid in the boiling tube could readily be inoculated with the micro-organisms under investigation. Cultures of micro-organisms, when introduced into either the fluid in the jar (*the outer fluid*) or that in the porous cylinder (*the inner fluid*), under suitable conditions, set up a chemical action, and the apparatus so far described constitutes a type of galvanic cell.

Fig. I



A, The cell.
B, Galvanometer.
C, Condenser.
D, Mercury cups to facilitate connections with different cells.
E, Morse key.

Throughout the main experiments platinum was used as the electrode, and to avoid the necessity of long platinum wires a short length of this metal was soldered with silver to a copper wire. For the purpose of insulation the wire was enclosed in a glass tube, the extremities of which were then fused round it, leaving a projection of platinum wire at one end and copper wire at the other, the former only being brought in contact with the nutrient fluid. Other forms of electrodes will be mentioned later. The leads from the electrodes were connected with a standard condenser of one micro-farad and the condenser discharged through a galvanometer by means of a Morse key. This arrangement was adopted, on the suggestion of Mr. H. Morris Airey, to eliminate the resistance of the circuit. A Clark's cell discharged in the same manner through the galvanometer served to show the number of deflections upon the galvanometric scale, which corresponded to the standard voltage.

The electrodes were carefully tested, and it was quite certainly ascertained that no E.M.F. was developed at the junction of the platinum and copper. The cell when charged was also thoroughly tested to determine whether any E.M.F. produced could be due to differences in temperature between the media separated by the porous cylinder, to osmotic effects, or to evaporation. It might be supposed that a possible difference of temperature between the junctions of copper and platinum might give rise to a thermo-electric effect, but this point was decided by heating the outer fluid several degrees above that of the inner, and under these conditions no E.M.F. was developed. Again, many trials were made, by varying the concentration of the solutions outside and inside the porous cylinder, to prove whether the E.M.F. could be produced by reason of osmotic effect, but this was found not to be the case. Evaporation from either the inner or outer fluids was also effectually prevented, and thus any E.M.F. which might be developed could not be attributed to evaporation currents.

As oxygen is not liberated in any of the reactions considered in this investigation, no electric effects could be produced by oxidation of the platinum electrodes.

Platinum, it is well known, often contains a local charge, and a difference of potential might thus be registered upon the introduction of the two electrodes into the solutions. Experience showed that this local charge occurred, but from repeated tests with control cells it was proved to be constant during the limits of an experiment, and allowance for this effect could be made. This relative charge upon the platinum wire, however, is altered by friction, and it is essential to prevent any shaking or other disturbance of the electrodes during an experiment. The leads from the electrodes were therefore fixed to a support, so that no disturbance of the electrode was possible when making the connections, and the difference of potential to be described could in no degree be attributed to friction inside the cell.

In every case the cells when set up were allowed to settle down and were tested before the commencement of any experiment. After these precautions against experimental error, the way seemed clear for a study of the actual effect produced by the vital activity of living organisms.

The fermentative activity of yeast upon solutions of sugar seemed to offer a promising subject for investigation, and ordinary commercial yeast in a very fresh condition, with glucose as a medium, was employed, because of the rapid and marked fermentation which is developed, and which one might expect to be reflected in a pronounced electrical effect.

For the study of the fermentation of yeast a very useful form of diaphragm can be constructed from parchment dialysing tubes, cut into convenient lengths. A glass cup tied firmly at one extremity and a glass ring at the other served to keep it distended, and as the tube could be discarded after each experiment, this kind of porous cylinder proved very convenient and was used in preference to a porous pot. The relative size of the cylinder and glass jar was such that when each contained 100 c.c. the level of the outer and inner fluids was approximately the same.

As an arbitrary standard it was found convenient to take 50 grm. of yeast and mix with 100 c.c. of water and to apportion 10 c.c. of this for each cell. By this method approximately the same quantity of yeast would be used for each cell, and the results of experiments would be comparable one with another.

The cell being set up, 100 c.c. of a given solution of glucose was poured into the cylinder and into the jar, the electrodes were inserted and the necessary connections made, and readings taken on the galvanometer determined the constant relative charge on the electrodes.

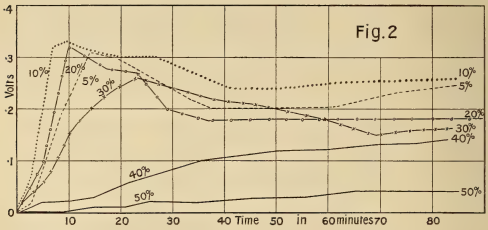
The yeast could now be introduced into the outer fluid, and after this was done readings of the galvanometer were taken at frequent intervals. For a short time after the introduction of the yeast no E.M.F. could be observed, but as the organism commenced its activity and the fermentation was set up, a gradually increasing voltage was registered, the rise of the voltage being determined by the concentration of the glucose solution, the temperature, and the quantity of yeast added.

On disconnecting the yeast-glucose cell, and substituting for it a standard cell, it was always found from the swing of the galvanometer that the glucose solution with yeast acted as the zinc of an ordinary galvanic cell, the current passing in the cell from the yeast-glucose solution to the glucose solution. Moreover, when the inner fluid was inoculated instead of the outer fluid, the seat of the chemical action became changed and the direction of the current was always reversed.

We may now proceed with some typical examples of the results obtained with experiments undertaken first with yeast.

### *Various Concentrations of the Medium.*

With the ordinary standard of 5 grm. of yeast at a temperature of 25° C. and a 10-per-cent. solution of glucose an E.M.F. of 0·32 volt was registered in the course of 7 minutes, after which the voltage gradually declined until at the end of 40 minutes it was 0·25, at which figure it remained fairly constant for a further period of 50 minutes. A reference to fig. 2 shows the variations of the voltage with solutions of different degrees of concentration.

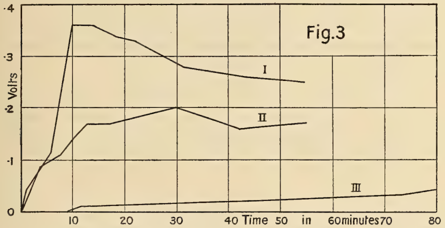


Curves showing development of E.M.F. in 5, 10, 20, 30, 40, and 50 per cent. solutions of glucose. 5 grm. of yeast employed in each case. Initial temperature 25° C.

With 20-per-cent. glucose the maximum voltage, 0·32, was reached in 10 minutes; with 5-per-cent., 15 minutes was occupied in reaching a maximum of 0·31. The 10-per-cent. thus appeared to be the best mean, but it will be seen that the concentrations of 5, 10, and 20 per cent., though varying slightly in their initial rapidity of action, attained a very similar voltage, and the curve thereafter followed much the same course. With 30-per-cent. glucose there was a slower development, the maximum voltage, which was 0·26, being attained only after 23 minutes, while with still higher degrees of concentration a markedly slower action and lower voltage indicated a solution much less favourable to the development of the yeast. Thus 40-per-cent. glucose registered only 0·18 volt after 90 minutes, and 50-per-cent. 0·08 after the same period.

### *Varying Amounts of Yeast.*

Fig. 3 gives the results of experiments to determine the effect of using different amounts of yeast. The three cells here given as an example were charged at the same temperature (23·5° C.) from the same solution of glucose, and to the first was added 5 grm. of yeast, to the second 2·5 grm., and to the third 0·5 grm. With 5 grm. of yeast the voltage rose rapidly to its maximum of 0·36 in 10 minutes. With 2·5 grm. the gradient of the curve was much more gradual, reaching 0·3 volt at the end of 2 hours. With the minimum of 0·5 grm. the curve rose very slowly; at the end of 80 minutes it only showed 0·04 volt, and did not reach the maximum of 0·3 volt until after a period of 3 hours. A comparison of these curves gives striking evidence of the greater speed of reaction corresponding to the more active fermentation, *i.e.*, the greater number of active yeast-cells present.



Curves showing development of E.M.F. with different amounts of yeast, in a uniform 10-per-cent. solution of glucose. I, 5 grm. of yeast; II, 2·5 grm.; III, 0·5 grm. Initial temperature 23·5° C.

### *Different Conditions of Temperature.*

Under different conditions of temperature the results vary in a corresponding manner. This is well exemplified by the curves shown in fig. 4. When the yeast is introduced into a 10-per-cent. glucose solution at 25° C. a voltage of 0·3 is attained in 9 minutes, and the maximum, 0·32, in 15 minutes. But at 17° C. a voltage of 0·3 is not reached until after 20 minutes, and the maximum, 0·32, after 25 minutes. At 10° C. the start was very slow, and there was a much more gradual approach to the maximum, 34 minutes being required for the development of 0·3 volt and 45 minutes for the maximum.

It is interesting to note that after the maximum voltage has been attained there is always a slight drop in the curve, which was not so pronounced following the more gradual rise at the lower temperature.

When cells were maintained at a temperature of 50° C. there was no deflection of the galvanometer, and when the temperature was lowered to 25° C. there was again no electric effect, showing that the vitality of the yeast had been destroyed. At 0° C. no deflection could be detected, but when the temperature of these cells was gradually raised by placing the cell in an incubator, a voltage was soon observed which continued to increase as the temperature became more favourable. At the freezing point therefore the yeast was not killed, and the absence of electrical action at this temperature shows an effect of suspended animation.

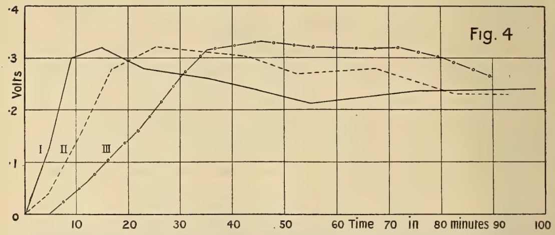


Fig. 4

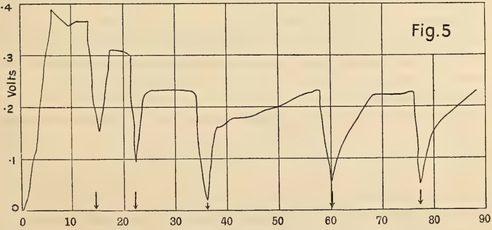
Curves showing E.M.F. developed at different temperatures. 5 grm. of yeast added in each case to a 10-per-cent. solution of glucose. I, initial temperature 25° C.; II, initial temperature 17° C.; III, initial temperature 10° C.

It should be mentioned that the initial temperature of the fermenting solution does not remain constant, but gradually changes as the process of fermentation proceeds.

These results demonstrate that the E.M.F. developed can only be attributed to the fermentative action of the yeast, and the electrical effects are the measure of the activity of the yeast. It has been shown that the character of the culture medium modifies to a great extent the electrical effects produced, and degrees of concentration which are too high for the favourable growth of the yeast are reflected in the diminution of the voltage registered and a much reduced speed of reaction. Further, the speed of reaction varies with the amount of active yeast present in the cells, and the gradient of the curve is directly influenced by this factor, being much steeper when the organism is employed in a more effective strength. It was also found that the gradient of the curve indicating the growth of potential is very steep when the yeast is introduced into a solution of glucose at the optimum temperature, but the curve is much less steep when the temperature approaches the limits of the maximum or minimum for the organism. At the minimum temperature, 0° C., and at the maximum, 50° C., no E.M.F. is produced, and it is important to note that a difference of potential is only found within the limits at which the various organisms can live.

The maximum voltage recorded with yeast and glucose or cane sugar was 0·3—0·4, and a voltage of this order was never exceeded in any of the experiments under any of the conditions tried. When battery cells of a larger capacity, containing 1½ to 2 litres, were used, it was found that no difference could be detected in the amount of the E.M.F. registered, and the voltage thus bears no relation to the volume of the fermenting liquor. It appears also that the voltage is quite independent of the thickness of the platinum wire or the surface of the electrode, thus when pieces of platinum foil up to 2½ cm. square were welded on to the platinum wire, the voltage remained the same.

A small current can always be detected in the yeast-glucose cells, and the voltage as registered by metallic conductors is due to the charge collected in the fermenting liquid. When a short circuit is made by joining the leads, the liquid in the neighbourhood of the electrodes is at once discharged, and the voltage is considerably reduced. But when the leads are again separated the E.M.F. gradually increases as the liquid recovers its charge through the activity of the yeast cells, and it becomes once more fully developed in a time varying according to the species of organism and the cultural conditions. Fig. 5 shows the effect of a short circuit in diminishing the E.M.F.



Time in minutes. Cell short-circuited at times indicated by arrows.

and the recovery of the potential difference after separating the leads. 5 grm. of yeast were used, and a 10-per-cent. solution of glucose at 28° C., the short circuits being made at intervals of 15, 22, 36, 60, and 78 minutes after the introduction of the yeast.

### *Enzymes.*

The important part which enzymes play in the activity of yeast during the breaking down of sugar into alcohol and $CO_2$ naturally leads one to an inquiry as to any electrical effects which may accompany their action, and to look for any such effects accompanying hydrolysis. At present I have had little opportunity for extended observations on this interesting question, but it may be worth while to record the results so far obtained. The two enzymes—invertase and diastase—with which some work has been undertaken, both exhibit distinct electrical response, though in a much smaller degree than has been already described.

*Invertase.*—For the study of invertase, a quantity of yeast was ground in a mortar with sand and lixiviated with a small quantity of water. After filtration through paper the filtrate was passed through porcelain to ensure that all yeast-cells were removed, and half of the solution was boiled to serve as a control.

Cells were charged with 5- and 10-per-cent. cane sugar, and the invertase solution added. In both cases a small voltage was registered, 0·02 for the 5-per-cent. and 0·03 for the 10-per-cent. The development of the E.M.F. was very gradual, and it was only after 70 minutes that the above readings were recorded. Treatment with Fehling's solution showed that inversion had taken place. No E.M.F. could be detected with the control cells to which the boiled solution was added, and tests with Fehling showed that the invertase had been destroyed.

*Diastase.*—A similar cell charged with a 0·5-per-cent. starch emulsion, to which was added 10 c.c. of a 5-per-cent. solution of diastase, gave an E.M.F. of 0·05 volt. The gradient of the curve of the potential development was again very gradual, a voltage of 0·3 being reached in ten minutes, after which it slowly increased.

Thus, in the hydrolysis of the polysaccharose starch to the monosaccharose glucose and fructose, a larger E.M.F. is produced than in the hydrolysis of the disaccharose cane sugar to the same monosaccharose.

### *Hydrolysis of Cane Sugar by means of Sulphuric Acid.*

For this experiment a cell was charged with a 1-per-cent. solution of sulphuric acid. It was found that if water were added to either the inner or outer fluid an E.M.F. might be developed, due to the variation of the concentration of the sulphuric acid on either side of the diaphragm, and to avoid this effect the cane sugar was added in the crystalline form. Following the addition of the sugar an E.M.F. was produced amounting only to 0·02 volt, the sugar and sulphuric acid being zincative. Testing with Fehling's solution showed that hydrolysis had taken place.

The small voltage developed when cane sugar is hydrolysed by dilute $H_2SO_4$ was further confirmed by adding the sugar as a thick syrup. In this case a battery of five cells in series was arranged, the temperature of the cells being 26° C. The syrup was added as quickly as possible to the cells, and readings taken at once. From this battery an E.M.F. of 0·1 volt was developed almost immediately, and too quickly to construct a curve showing the times of development of the E.M.F. This method, however, is useful in showing how the hydrolysis proceeds and the speed with which this reaction dies away.

### *Bacteria.*

I may now briefly refer to some experiments which were made with certain species of bacteria. Pure cultures of *Bacillus coli communis*, *B. fluorescens*, *B. violaceus*, and *Sarcina lutea* were obtained from Dr. Kral, and, after sterilisation as previously described, inoculations were made from each of these micro-organisms upon nutrient solutions in the boiling tubes. The solution used was a modification of the well-known Pasteur's solution, viz.:—

| | |
|---|---|
| Potassium phosphate ............ | 2 |
| Calcium phosphate ................ | 0·2 |
| Magnesium sulphate ............ | 0·2 |
| Ammonium tartrate................ | 10 |
| Asparagine ......................... | 0·5 |
| Water ................................ | 1000 |

*B. coli communis* flourished readily in this medium and proved to be an excellent subject for the investigation. At 30° C. this organism, when grown in the above solution, developed an E.M.F. recorded by the galvanometer as 0·308 volt. Using the same solution, but replacing the asparagine with 0·2-per-cent. starch, it gave rise to a voltage of 0·349. At 20° C. the voltage recorded was 0·534.

In the case of *B. violaceus*, *B. fluorescens*, and *Sarcina lutea* no E.M.F. was produced, these organisms causing no deflections of the galvanometer, either at 30° C. or at 20° C. It was found that the medium employed was unsuitable for the growth of these bacteria, and they had quite failed to develop under these conditions.

At this stage the action of yeast was also studied, and this proving a very convenient substance for experiment, further investigation of bacteria was for the present abandoned.

The employment of this form of galvanic cell may be usefully extended to the observation of other physiological processes, and it will be of interest to note some electrical effects indicated during the conversion of venous to arterial blood and *vice versâ.*

A quantity of fresh warm blood was obtained, a part of which was poured into a parchment tube and part placed in the jar surrounding it. With a platinum electrode in the jar and in the parchment tube, it was found that arterial blood was zincative to venous blood. Many trials made, by alternately forcing air or carbonic acid through the blood in the jar or in the tube, showed that the oxygenating stream raised the potential of the blood, so that an electric current passed in the cell from the arterial to the venous blood; with the entering stream of $CO_2$, however, the potential was lowered and an electric current was produced in the opposite direction. Thus the arterial and venous bloods possess a contrary electrical sign. As a corollary it would seem that this change of sign may play an important part in the electro cardiogram of the heart, and that when arterial is converted into venous blood this change of sign may be looked for as one of the accompanying events.

### *Comparison with a Galvanic Cell.*

The study of the electrical effects during well-known chemical actions by means of a galvanic cell constructed on the principle already described is of much interest for comparison with the electrical effects of fermentation.

A glass jar containing a porous pot, into each of which a dilute solution of sulphuric acid is poured so that the level of the outer and inner fluids should be the same, a pair of platinum electrodes inserted one in the jar and one in the porous pot, and a little zinc added to either the outer or the inner fluid, constituted a cell comparable with the glucose-yeast cell previously described.

With such a cell, using a 10-per-cent. solution of sulphuric acid and 5 grammes of granulated zinc, the condenser and galvanometer gave an E.M.F. of 0·7 volt (see fig. 6). The direction of the current in the cell was from the sulphuric acid and zinc to the sulphuric acid, and thus followed the same course as that observed in the case of glucose and yeast. The curve indicating the E.M.F. in volts is also developed in a manner parallel to that of the yeast and glucose. The galvanometer showed no immediate effect following the introduction of the zinc, but during an interval of 8 minutes the maximum voltage was gradually developed. This voltage died away by degrees as the zinc was dissolved and the chemical action ceased.

It should be noted that the zinc was not allowed to come in contact with the platinum electrodes, and thus the bubbles of hydrogen escaped from the zinc and not from the platinum wire.

A similar cell, in which hydrochloric acid was used in conjunction with zinc placed in the jar, gave parallel results, the voltage being 0·7.

Other substances used to develop an E.M.F. were phosphorus, potassium, and sodium.

*Phosphorus.*—The cell and porous pot were charged with distilled water, and a pair of platinum electrodes, one immersed in the outer and the other in the inner fluid, were connected with the condenser and galvanometer. The relative charge upon this pair of electrodes being determined, a stick of phosphorus was suspended partially immersed in the outer fluid, the temperature of the water in the jar being 10° C. For a period of 28 minutes no effect could be observed on the galvanometer, the constant relative charge upon the pair of electrodes giving the same number of deflections, although copious fumes were being given off. At the end of this period the effect of the oxidation could be observed, and the voltage developed steadily increased. After 11 minutes it increased to 0·05, after 13 minutes to 0·06, and after 35 minutes to 0·1. After this time, the phosphorus, becoming dry, ignited, and the E.M.F. then suddenly increased to 0·21 volt. It should be noted that the current was in the same direction as with zinc and sulphuric acid, namely, the oxidising phosphorus was zincative.

In this experiment the comparatively long period between the introduction of the phosphorus and the first signs of the development of any E.M.F. was due to the low temperature of the cell (10° C.), and with a higher temperature it was found to be considerably reduced.

*Potassium and Sodium.*—A similar cell, with water in both jar and porous pot and with a pair of platinum electrodes, gave an E.M.F. when either metallic potassium or sodium was introduced into the jar, the direction of the current being the same as in the other cases already mentioned.

From the curves given it will be noted that a short interval of time elapses after the introduction of the zinc into the sulphuric acid before the E.M.F. can be detected, although the action of the acid upon the zinc is at once evidenced by the liberation of the hydrogen or the fumes given off from the phosphorus. A similar time of presentation was observed with both sodium and potassium. Although experiments have not been undertaken with special regard to this point, it is suggested that the gradient of the curve of the E.M.F. is a function of the temperature.

Thus, a study of this cell when charged with dilute sulphuric acid and zinc, or with water and either phosphorus or potassium or sodium, gains importance from its bearing upon the action of yeast and glucose in a similar cell. It has been proved that the principle of this special type of cell is equally applicable to the two cases, and the results obtained are precisely analogous in both sets of experiments. For the purposes of the present research it is sufficient to have established this point, to note that an E.M.F. is developed in these special cells, and that the centre of chemical activity with platinum electrodes is always zincative.

### *Non-polarisable Electrodes.*

The behaviour of non-polarisable electrodes in a cell of this construction is also of much interest, and an exact parallel is found between the action of these electrodes when used in conjunction with either a yeast-glucose or a zinc-sulphuric acid cell.

The non-polarisable electrodes consisted of a glass **U**-tube, plugged at the bend with kaolin; into one limb of this tube a saturated solution of zinc sulphate was poured and a rod of amalgamated zinc inserted, the zinc being connected with the condenser and galvanometer leads. Into the other limb was placed a solution of glucose or of sulphuric acid of the same concentration as that employed in the cell, and a lamp wick soaked in the glucose or sulphuric acid solution was arranged so that one end of it dipped into the **U**-tube and the other into the cell.

Another form of non-polarisable electrode consisted of a pair of glass tubes, closed at one end either with a plug of kaolin or with a piece of bladder tied tightly round. These tubes were partially filled with the zinc sulphate solution, into which dipped a rod of amalgamated zinc. One of the tubes was inserted into the outer and the other into the inner fluid of the cell, and the connections were made with the condenser-galvanometer circuit as before.

Such electrodes, when used either with a yeast-glucose cell or a zinc-sulphuric acid cell and the condenser, gave no deflections upon the galvanometric scale. In both these cases, however, the existence of an electric current, depending upon the resistance of the circuit, can be shown by connecting the leads from the non-polarisable electrodes directly with the galvanometer. The electrical action is developed both in the yeast-glucose and in the zinc-sulphuric acid cells, as shown by the current generated, but the condenser method is not applicable with the use of non-polarisable electrodes.

As a further test of this principle, cells were set up with a pair of platinum electrodes and a pair of non-polarisable electrodes. With this arrangement four possible combinations of the electrodes are possible:—

I. A pair of platinum electrodes.
II. A pair of non-polarisable electrodes.
III. A platinum electrode in the jar and a non-polarisable one in the porous pot.
IV. A non-polarisable electrode in the jar and a platinum one in the porous pot.

With a zinc-sulphuric acid cell, in which the constancy of the relative charges upon the four pairs of electrodes had been determined, the following facts were observed when the zinc was added to the sulphuric acid in the jar:—

I. Platinum-platinum electrodes. After a short interval an E.M.F. was developed which soon attained its maximum, and then gradually died away as the zinc was consumed (fig. 6).

II. Non-polarisable electrodes in both jar and porous pot. No E.M.F. was developed; that is, the discharge of the condenser through the galvanometer

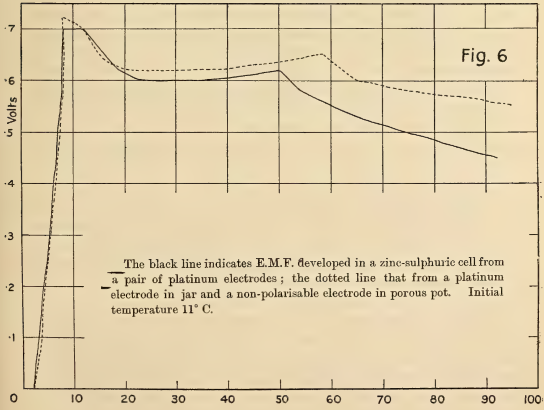


Fig. 6

The black line indicates E.M.F. developed in a zinc-sulphuric cell from a pair of platinum electrodes; the dotted line that from a platinum electrode in jar and a non-polarisable electrode in porous pot. Initial temperature 11° C.

produced no effect. When, however, these electrodes were connected directly with the galvanometer the spot immediately moved "off scale," showing the existence of an electric current developed in the cell, the strength of which depended upon the resistance of the circuit.

III. Platinum in the jar, non-polarisable electrode in the porous pot. An E.M.F. was developed of much the same voltage as in I, as shown by a comparison of the curves in fig. 6.

IV. Non-polarisable electrode in the jar and platinum in the porous pot. No E.M.F. could be detected with the condenser, but an electric current was evident as in II.

It should be remarked that the relative charge upon the electrodes in the cases III and IV was, relatively speaking, large; thus in III the number of deflections showed a difference of potential of 1 volt, and also in IV a difference of potential of 1·1 volt. The deflections on the scale of the galvanometer were of opposite sign, that in III being anti-zincative, and that in IV zincative. In III the voltage due to the relative charge decreased as the zinc was acted upon by the sulphuric acid, at first very rapidly, until the maximum E.M.F. was produced by the zinc and sulphuric acid. Following this it increased gradually as the action ceased; and after some 12 hours, when all the zinc had been dissolved, the relative charge showed only a slight difference from its former voltage. In this case the E.M.F. produced by the solution of the zinc was opposite in sign to the relative charge of the pair of electrodes.

In IV the relative charge remained constant throughout the duration of the experiment.

A cell charged with yeast and glucose and a similar four pairs of electrodes gave results exactly parallel with those described for zinc and sulphuric acid:—

I. Platinum electrodes. The curve of the E.M.F. with galvanometer and condenser developed in the normal manner.

II. Non-polarisable electrodes. No deflections of the galvanometer were to be observed when the condenser was discharged through it, but a very distinct current was registered when the electrodes were connected directly with the galvanometer.

III. This gave the normal curve of the E.M.F. as in I.

IV. The same effects as in II were indicated.

All these tests combine to show quite clearly that, though a current is certainly generated, no voltage can be registered with non-polarisable electrodes, and this special form of electrode is unsuitable for use with the condenser.

*Other Electrodes.*—Although platinum wires were employed as the standard electrodes throughout this investigation, trials were also made with other metallic wires such as gold, nickel, tin, zinc, and aluminium. All these metals when used as electrodes, as well as carbon plates from Leclanché cells, showed the existence of a difference of potential between the yeast-glucose solution and the glucose solution, and also the existence of an electric current passing in the cell from the yeast-glucose to the glucose. The amount of the E.M.F., however, was found to vary with the different electrodes used; but an inquiry into the causes of this variation leads into the domain of physical chemistry and lies outside the scope of the present investigation.

It may be mentioned that a battery of six cells with carbon electrodes connected in parallel gave a current of 1·25 milliampère.

In this preliminary investigation only commercial yeast of the kind known as "German yeast" was used, and at present no experiments have been made with pure cultures of the different races of *Saccharomyces.* So far cane sugar and ordinary glucose dissolved in distilled water have provided the nutrient media for the yeast, but evidence is not wanting that very slight differences in chemical composition directly affect the character of the results obtained. Enough has been done to show that this electrical method may be conveniently utilised to study the vital activity of yeast and other organisms when growing under special cultural conditions. It will be a matter of interest to compare the E.M.F. produced by the different races of *Saccharomyces* in the various kinds of sugars, and also to study the effect of inorganic salts, such as phosphates, nitrates, &c. To follow up the question as it affects enzymes opens up a large field of work, and this subject presents a problem in itself. The action of bacteria in producing electrical effects also awaits more detailed investigation.

I desire to express my thanks to Dr. A. D. Waller for the interest he has shown in this research and for the opportunity he kindly afforded me of repeating my results in the Physiological Laboratory of the University of London. My thanks are also due to Dr. W. M. Thornton for the kind help he has given me in the revision of the proofs.

### *Summary and Conclusions.*

The disintegration of organic compounds by micro-organisms is accompanied by the liberation of electical energy.

The difference of potential was investigated by the employment of a special type of galvanic cell with platinum electrodes, by means of which the electrical charges set free in the vital processes of the micro-organisms were collected and transferred to a condenser. Since $CO_2$ is the only gas liberated in alcoholic fermentation there is assumed to be no oxidation of the platinum electrodes. The charge, as measured by a ballistic galvanometer, was found to correspond to an E.M.F. of 0·3 to 0·5 volt between the fermenting and non-fermenting fluids. A difference of potential also occurs during hydrolysis, either by enzymes or by weak acids.

Various metallic electrodes can be used in conjunction with a condenser for the estimation of the E.M.F. produced. Non-polarisable electrodes, however, give no voltage with a condenser and are unsuitable for this method. Distinct electric currents are found whichever form of electrode is used.

The electrical effects are an expression of the activity of the micro-organisms and are influenced by temperature, concentration of the nutrient medium, and the number of active organisms present. These effects are only found within the limits of temperature suitable to the micro-organisms and under conditions which are favourable to protoplasmic activity.

The maximum voltage recorded was 0·3 to 0·5 volt, and a voltage of this order was never exceeded in any of the experiments undertaken with micro-organisms.

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# *Fractional Withdrawal of Complement and Amboceptor by Means of Antigen.* (*Preliminary Note.*)

By J. O. Wakelin Barratt, M.D., D.Sc. Lond., Director of Cancer Research Laboratory, University of Liverpool (Mrs. Sutton Timmis Memorial).

(Communicated by Prof. C. S. Sherrington, F.R.S. Received May 15, 1911.)

The problem which forms the objective of the present investigation is the determination of the extent to which complement and amboceptor are withdrawn by amounts of antigen which are insufficient to cause complete removal of these two substances.

In this investigation the source of both complement and amboceptor was normal human blood serum, which was employed alone when it was desired to preserve the natural relationship of complement and amboceptor; when it was desired to vary this relation, artificial mixtures of complement and amboceptor were prepared, which presented wide divergence from the relation obtaining in normal serum.

Human serum has a hæmolytic action, not very considerable in degree, upon the red blood cells of the rabbit, which were employed as antigen. In all experiments made with this hæmolytic system the same volume of fluid was employed. Estimations of complement and amboceptor were expressed in terms of the equivalent quantities of active and inactivated normal serum respectively, it being found that the composition of healthy sera ordinarily exhibited only slight variation, any marked change in the content of these two substances being exceptional.

The estimation of complement was sometimes made by determining the maximum amount of red blood cells completely laked by a given quantity of complement-holding fluid in the presence of a considerable excess of amboceptor, and then ascertaining the amount of normal serum capable of producing the same degree of hæmolysis under similar conditions of experiment. It was, however, sometimes found more convenient to keep the amount of red blood cells employed constant, and to vary the conditions of experiment until these were so adjusted that complete hæmolysis was observed at the end of a period of 90 minutes at a temperature of 37° C.

The estimation of the amount of amboceptor in serum was carried out by first determining the amount of complement present and then ascertaining, in the absence of any addition of amboceptor, the hæmolytic power of the serum. The latter was then compared with the hæmolytic power of a number of admixtures of complement and amboceptor, in which the content of complement was the same as in the serum tested, but the amounts of amboceptor present formed an increasing series, the comparison being made under similar conditions of experiment. The quantity of amboceptor is represented by the amount contained in that admixture which exhibited the same hæmolytic power as the serum under consideration.

Before determining the extent to which partial withdrawal of complement and amboceptor by subminimal amounts of antigen occurs, it was necessary to study the effect of variations in the amounts of complement and amboceptor in respect of hæmolytic power. An investigation involving this inquiry has been carried out with the aid of a heteroamboceptor by Kiss,* who determined the varying amounts of complement and amboceptor which were just capable of producing complete hæmolysis of the same amount of red blood cells. This author observed that as the complement was increased in amount the quantity of amboceptor required became diminished. In our own experiments, in which the employment of a heteroamboceptor was avoided, both complement and amboceptor being derived, as already mentioned, from the same source, a similar relationship is also exhibited in a quantitative form. When a normal serum is employed the extent to which a disproportion of complement and amboceptor can be produced is limited by the relative feebleness of the serum. If, however, an immune serum is used, wider divergences in the amount of complement and amboceptor employed may be obtained.

If, in normal serum, fractional withdrawal of complement and amboceptor by means of antigen is carried out, it is found that these two substances are withdrawn in the proportion in which they naturally exist in the serum, so that the liquid remaining after partial withdrawal has been effected is comparable, as far as complement and amboceptor are concerned, to diluted normal serum.

If, instead of employing normal serum alone, a mixture of normal active serum (complement + amboceptor) with inactivated normal serum (amboceptor) is used, so that a liquid is obtained in which the concentration of amboceptor is increased, while, at the same time, the concentration of complement is reduced, it is found that complement and amboceptor are at first removed by subminimal amounts of antigen in approximately the same relative proportion in which they exist in the mixed sera, the amount of amboceptor being, relatively to the complement, removed in excess. If this relationship is studied more closely it is seen that the actual quantity of amboceptor removed is greater than that withdrawn by the same amount of antigen from a similar concentration of amboceptor afforded by active normal serum, while the actual quantity of complement removed is less than would be withdrawn had active normal serum been employed.

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* "Untersuchungen über die Fermentnatur des Komplementes," 'Zeitschr. f. Immunitätsforschung,' 1909, vol. 3, p. 558.

If normal serum from which amboceptor has been removed by means of antigen acting at 0° C. is added to inactivated serum (amboceptor), so that a mixture is produced in which complement is in excess, it is found that fractional withdrawal of complement and amboceptor proceeds in a similar manner, relatively more complement than amboceptor being removed. If the actual quantities withdrawn are considered, it is found that the amount of complement taken up by a given amount of antigen is greater than, and the amount of amboceptor less than, would have been removed had active normal serum been employed to produce the same concentration of amboceptor.

The mode of fractional withdrawal of complement and amboceptor by antigen is not such as would be expected if a simple stoichiometric relation between these three substances existed. Whatever the ultimate nature of the reaction in question may be, in the early stages of the process, at any rate, no fixed relationship between the quantities interacting exists.

When fractional removal of complement and amboceptor takes place in an artificial admixture of these two substances, the relation between the complement and amboceptor removed approximates at first to that obtaining in the admixture, provided the amounts removed are small. If relatively large amounts of complement and amboceptor are removed by antigen, the divergence between the two, though still present, tends to become less marked. In consequence, the difference between the complement and amboceptor still remaining becomes less pronounced, and, as withdrawal proceeds, tends to approach the relationship obtaining in normal serum. By using in these experiments complement and amboceptor contained in the same serum and thus avoiding the employment of a hetero-complement (by which is meant a complement which is heterologous in respect of the amboceptor employed), it becomes possible to compare the relation of complement and amboceptor in the liquid remaining after fractional withdrawal of these substances from an admixture with that obtaining in normal serum. It is seen that, as withdrawal proceeds, the liquid remaining tends to become more and more closely comparable, in respect of complement and amboceptor, with diluted normal serum.

# *The Influence of Ionised Air on Bacteria.*

By W. M. Thornton, D.Sc., D.Eng., Professor of Electrical Engineering at Armstrong College, Newcastle-upon-Tyne.

(Communicated by Sir Oliver Lodge, F.R.S. Received June 20, 1911.)

[Plates 7—12.]

1. On exposing surface films of bacteria upon agar to the electric wind from charged needle points, with subsequent incubation, it is found that the wind from negatively charged points has more bactericidal effect than that from points positively charged.

In a recent paper* it was suggested that there should be this difference in consequence of the positive charge found to be associated with fresh vegetable cells, and further, that negatively charged air might prove useful in the treatment of tuberculous disease of the lung by retarding the growth of the bacteria. Before attempting direct trial of the latter point it was necessary to show that negative electric charge does inhibit bacterial growth in general under laboratory conditions of culture, and the present work was undertaken for this purpose.

2. The most suitable method of ionising air on a large scale either positively or negatively is by unidirectional point discharge at high potential. The voltage gradient should be such that no sparks pass, but that a faint blue glow is seen at each point, indicating the space in which most of the ionisation is taking place. The glow at the negative point is larger than that at the positive, and begins at a lower pressure. In either case ions of the same sign as that of the point are repelled, causing the electric wind. This was, with the pressures used in the present case, perceptible on the palm of the hand at a distance of about 10 cm.

The discharge can take place into free air, and the circuital flow is then completed either by diffusion of the ions to oppositely charged surfaces which are under the influence of the electrostatic machine used, or by recombination in the air. The latter is known to occur very rapidly in certain cases. In order, therefore, to expose the germs to a continuous and even wind for long periods, it was necessary to keep the machine running, and at the same speed, for the whole time of exposure.

3. The first arrangement of discharging points, that used in obtaining the results in figs. 7 to 10 (Plates 7 and 8), is shown in fig. 1. Two bell-jars,

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* "On the Opposite Electrification produced by Animal and Vegetable Life," 'Roy. Soc. Proc.,' B, 1910, vol. 82.

5 inches diameter and 10 inches long, with stoppered openings at the top, were supported on ebonite and glass insulators. The discharging points, three fine steel sewing needles, were attached to a copper wire passing through the stopper to an electrostatic machine, one of the jars being connected in this way to the positive terminal, the other to the negative. The opposite pole in each case was connected by a wire entering through a central hole in the base plate to a thin metal disc, upon which was placed a Petrie dish containing agar sown upon the surface with an emulsion in water of the organism to be exposed. The distance of the points from the agar was in no case less than a centimetre.

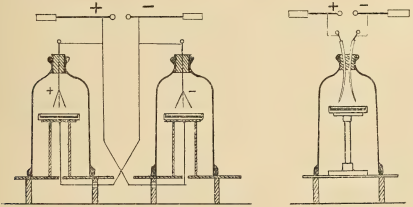

Fig. 1 Fig. 2

Fig. 1.—Discharging points in separate vessels.
Fig. 2.—Discharging points over the same plate.

The glow at the points could just be seen in diffused daylight. The photograph of fig. 3 (see at bottom of Plate 12), taken with the needles illuminated by a Nernst projector lamp, shows the proportions of the group and the volume of the negative glow in each case. From the photomicrograph (fig. 4) the negative glow starts as a regular cone, drawn here to one side by the proximity of the positive pole, and the positive glow (fig. 5) appears on the side of the needle, in this case sharpened by a file cut. The former has a vertical angle of 135°, converging upon a point about 0·15 mm. outside of the surface of the needle.

The radial length of the glow is 0·10 mm., and its volume about 0·90 cu. mm. The positive glow is irregular and extended over a larger area on the needle than the negative, though the luminous volume is only about one-half that of the latter. With the arrangement shown in fig. 2 the current was 4·4 micro-ampères in both the positive and negative leads. At the negative pole the rate of ionisation, assumed to all take place in the glow, is from the above figures $3 \times 10^{13}$ charges, each of the magnitude $e$ ($4{\cdot}6 \times 10^{-10}$ E.S.U.), per cubic millimetre per second. The photograph (fig. 6) shows a faint luminous line proceeding straight outwards from each of the positive points, distinct from the ordinary visible glow. This is only found after long exposure—three-quarters of an hour—of the photographic plate. It is possible that this is the space in which negative ions are produced and receive inward acceleration in positive point discharge. Some of these would be, no doubt, carried away by the positive wind, and this might account for the presence of negative ions in positive point discharge observed by C. T. R. Wilson and by N. R. Campbell. The luminosity may also be from ionisation produced by "Entladungstrahlen."* No such line is to be seen at the negative poles. The length is here 8 mm. At both poles there is a bright luminous patch on the surface of the metal, but at the positive point there is also some scintillation, possibly due to the negative bombardment, which is not seen at the negative point. The voltage used was not directly measured, but gave sparks 2 to 2·5 mm. long between 1·5 cm. diameter brass discharge knobs. On open circuit, that is, with the needle points disconnected, the machine gave 1·5 cm. sparks, running at the same speed as before.

4. The second arrangement of discharging points, that used in obtaining figs. 11 to 15 (Plates 9 and 10), differed only from the first in having both positive and negative points brought into the same jar, as in fig. 2. The needle points emerged 2 mm. from the leading-in glass tubes, and were directed to positions on the plates 2½ cm. centre to centre. The stand holding the exposed plate was supported by a sulphur rod, attached to an ebonite base-plate. The leak to earth other than from point to point was exceedingly small. The object of having both points over the same culture was to obtain comparison of exposures in which the current passing was the same for both. The outer part of the agar surface provided a control, which for short exposures may be taken as unexposed, though in certain cases with prolonged exposure the whole plate is cleared. With the two points discharging on to the same surface the action was much stronger than in the first arrangement.

5. Of the organisms exposed in the first way (fig. 1) photographs of typical exposures are given of *B. anthracis*, *B. pyocyaneus*, *Sarcina lutea*, *Pneumococcus*; and in the second (fig. 2) of *B. coli communis*, *B. Friedländer*, *B. typhosus*, *B. asiaticæ choleræ*, *B. dysenterica Shiga*. These were all fresh, active growths, though most of them had been often sub-cultured.

It was found that there was a marked difference between the sensitiveness of the bacteria examined. On account of the use made by previous experimenters of *B. anthracis*, *B. typhosus*, and *B. coli communis*, these were used in determining the best conditions of exposure.

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* *Vide* Sir J. J. Thomson, "Conduction of Electricity through Gases," §§ 242 and 310.

In fig. 7 (Plate 7) the organism is anthrax exposed for 20 minutes, 10 minutes' exposure failing to show any effect. The colonies in the control are large and uniformly distributed. In the central (positive) plate they are cleared opposite two points, the third has missed fire. In the negative plate the cleared areas are somewhat larger and the colonies fewer. In fig. 8 the exposure was for 30 minutes, and the influence of time and of difference of sign of charge is now unmistakable. The positive plate is well cleared, the negative contains one colony only, and at a corner where the wind is least likely to have taken effect. In fig. 9 (Plate 8) with 50 minutes' exposure of a dense sowing the positive plate shows little growth, and on the negative there is one colony. Many hours' exposure are necessary to completely sterilise the plates with certainty in this way. Fig. 10 is of Pneumococcus, 1 hour 10 minutes' exposure. From a very dense sowing only about a dozen colonies developed on the positive plate and a few rudimentary ones on the negative.

In the case of *B. pyocyaneus*, exposed as in fig. 1, the negative plate was entirely cleared in two and a-half hours, the positive about one-half. The control showed a dense growth. This may be regarded as a sensitive organism, though not to the same degree as *B. asiaticæ choleræ*, given later.

*Sarcina lutea*, exposed for two and a-half hours, gave nearly as many colonies on the positive plate as on the control, the negative having only a few small ones.

6. On account partly of the long time sometimes taken to produce a distinctive difference, the arrangement was changed to that shown at fig. 2. The first exposure of *B. coli communis* was for 18 hours. On examining the dish it was found that the discharge had dried the jelly, forming two shallow pits, the smaller caused by the positive discharge, the larger by the negative. The angle of the positive cone was one-half that of the negative.

The *B. coli communis* series is given in fig. 11 (Plate 9), fig. 13 (Plate 10), and fig. 18 (Plate 11), the exposures in Plate 9 being $1\frac{1}{4}$, $4\frac{1}{2}$, and 18 hours respectively. A good deal is cleared in the first, all but a small patch in the bottom corner of the second, and the third entirely so. Fig. 12 is of *B. typhosus*, the times of exposure being 1 hour, $\frac{1}{2}$ hour, and $2\frac{1}{4}$ hours. There is a trace of clearing in the $\frac{1}{2}$-hour plate, none at the positive in 1 hour, though a good deal at the negative, and in $2\frac{1}{4}$ hours an almost clear plate.

The two upper photographs, fig. 13 (Plate 10) are of *B. coli communis*, the right hand exposed for 1 hour, the left for ½ hour, in the latter of which the signs of clearing are slight.

The organism most sensitive to the point discharge of any so far examined is *B. asiaticæ choleræ*, two exposures of which are given in fig. 14 (Plate 10), that to the left having been exposed for a quarter of an hour, that to the right for half an hour. This bacterium is also sensitive to light, dying out in a few days in diffused daylight.

In the case of *B. dysenterica Shiga*, fig. 15 (Plate 10), half an hour had little or no effect, the whole surface being covered with fine densely packed colonies. In an hour, however, most of the plate was cleared. This and the above result with *B. asiaticæ choleræ* may be compared with the observation of Buchner, that the growth of *B. typhosus* is prevented by exposing a freshly-sown culture for an hour to full sunlight.

From the above results it may be concluded that: (1) air ionised by either positive or negative point discharge has a strong bactericidal action; (2) the negative discharge is much more effective than the positive for short exposures, though the result after many hours' exposure is nearly the same for both. It is possible that some part of the bactericidal action of the positive wind is owing to negative ions produced at the positive point.

7. Since oxygen is electro-negative and ozone is known to be produced by electrical discharge, the electric wind was examined for ozone by paper which had been moistened with a solution in alcohol of tetra-methyl-*p.p.*-diamido-diphenyl-methane.* This has the property of turning violet in the presence ozone, yellow with nitric oxide. On exposure to the discharge arranged as in fig. 2, it was found that ozone was produced at the negative point and to a much less degree at the positive. There was no yellow coloration, though the paper dried a purplish brown. The presence of ozone in the wind suggested the possibility that the bactericidal action might only be indirectly the result of ionisation, that the well-known sterilising influence of ozone (whatever the ultimate cause of that may be) might explain the facts equally well. To decide this, exposures were made in nitrogen and pure hydrogen· The former was, however, found to contain 0·8 per cent. of oxygen, and since nitrogen when mixed with oxygen even in small quantities cannot be regarded as quite inert, the result given in fig. 20, obtained in it using *B. typhosus*, is chiefly of interest in showing that the effect is of the same order of magnitude as in hydrogen and air. In the case of hydrogen the bell-jars were well exhausted and filled with the gas several times before exposure, with the

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* See Fischer and Braemar, "On the Production of Ozone by Ultra-violet Light," 'Ber.,' 1905, vol. **38**, No. **3**, p. 2633.

Petrie dishes in position containing the bacteria. The photographs of Plate 11 of *B. asiaticæ choleræ*, fig. 16; *B. typhosus*, fig. 17; and *B. coli communis*, fig. 18; in hydrogen, with the discharging point arranged as in fig. 19, show that the sterilising influence of the negative discharge is somewhat greater in hydrogen at atmospheric pressure than in nitrogen or, by comparison with the previous results, than in air, owing possibly to the greater velocity and range of ions in hydrogen. It would, however, appear that in these cases the nature of the gas is not of the first importance, and that it is the presence of the electrical charge which is the chief inhibiting cause.

The criticism having been made that water-vapour from the surface of the agar might give rise to the formation of hydrogen peroxide in the glow, trials were made with the exposure vessel arranged as in fig. 19. The wire to which the discharging needle is soldered is led down a glass tube through which a stream of the gas to be used is slowly passed. The needle is held in a central position by a glass bead *b*, and the gas, ionised by the discharge, is blown gently upon the agar surface sown with bacteria. The Petrie dish rests on a metal plate connected to the positive pole. There is no possibility of water-vapour reaching the needle point, and the arrangement is very convenient for the examination of the effect of ionised gases on surface cultures. In operation the outlet from the jar was first sealed with paraffin wax and the

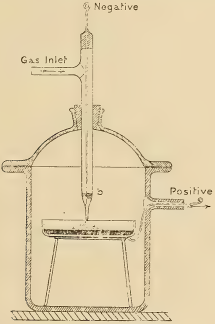

Fig. 19.—Discharge point in tube.

vessel exhausted and filled several times with hydrogen passed through sulphuric acid and tubes of soda-lime. An opening was then made by passing a hot wire through the wax, and a continuous stream of gas sent past the electrified needle from a cylinder of hydrogen. Fig. 18 (Plate 11) shows the result of exposing plates of Conradi and Drigalski litmus lactose medium sown with *B. coli communis*, which were before exposure inverted and dried in a warm chamber to fix the emulsion on the surface. The jet of hydrogen without ionisation was passed for three-quarters of an hour over one plate, which was then removed. The electrostatic machine was then started and the other plate exposed for the same time to the same stream of hydrogen, now negatively ionised. The influence of the electrification is marked, the area beneath the nozzle being quite cleared. This experiment is also of interest in showing that pure dry hydrogen has no inhibiting effect on bacterial growth. The conditions in this test were the most stringent that could be devised. The surface of the agar was firm and dry and the time of exposure short for so insensitive an organism as *B. coli communis*. The plate may be compared with fig. 13, where the exposures were for half and one hour using the same bacillus.

The exposures in nitrogen and hydrogen were made in the Pathological Laboratory of the University of Durham College of Medicine, under the direction of Professor Hutchens, to whom, with his colleague, Dr. P. Laws, the author is gratefully indebted.

8. When bacteria are killed by any physical or chemical agency it is generally accepted to be in consequence of the coagulation of protoplasm. In the present case there are two immediate possibilities, apart from direct electrical action, by which this might be achieved. The effect may be due to the influence of ultra-violet light from the glow, or the mechanical bombardment by the wind might be sufficient to produce inhibition of growth such as is known to be caused by mechanical vibration. To put the first to the test a piece of optical quartz, 2·4 cm. square by 1 mm. thick, was laid on the surface of the agar, which had previously been sown with a strong emulsion of *B. asiaticæ choleræ* as a sensitive indicator. The dish was then exposed for half an hour in such a position that the negative needle point was central with the quartz square and at 1 cm. above it. The positive point was then about 1·5 cm. from the edge of the square. At the end of the exposure the quartz was removed, care being taken to prevent liquid from the edges flooding into the space occupied, though some unavoidably ran over the space under the positive needle. The result on incubation is given on the right of fig. 21. The growth on the part covered by the quartz is denser than elsewhere, the space around showing evident signs of clearing.

It may then be concluded that the bactericidal effects observed in the photographs are not due to the influence of ultra-violet light, bactericidal rays of which would freely penetrate the thickness of air and quartz used.

In order next to shield the organisms from the direct wind by interposing a stout membrane between the point and the agar, a plate was prepared and sown upon which a cigarette paper was laid moistened with the emulsion. The discharging points were arranged to be on the centre line of the paper, and the exposure was, as before, half an hour. The effect, also given in fig. 21, shows that the negative discharge penetrates the moist paper, though it is not so active as without the paper. This result, that the destructive influence can penetrate a membrane like wet paper in contact with bacteria, is of importance in showing that the electrical charge of ionised air may be expected to pass through lung membranes into the blood.

There is, however, in the present case a third possibility, that the action is not caused by the ions carried with the electric wind but by some penetrating radiation independent of it. To test this a light wire frame was made to hold a cigarette paper, dry or moistened as before, 3 mm. horizontally above the surface of the agar, which had been sown as usual. The result was that there were no cleared areas under the points. The whole effect may therefore be attributed to the direct influence of, and contact with, ions in the electric wind.

9. It was shown in the previous paper that both red corpuscles and leucocytes have a strong negative charge. On the other hand, fresh bacteria have a charge positive in sign and of the same order mass for mass as that of the blood cells.

The chief function of leucocytes is known to be that of absorbing bacteria from the blood. Their thin walls are easily pierced, but the origin of the force necessary for this to take place has not been located. The general conception of chemiotaxis, covering all movement in response to chemical stimulus, has been the closest approximation. It is now suggested that the initial stimulus to the process of absorption may be more simply explained by the attraction of the positively charged bacteria by the large negatively charged leucocytes. In the case of fresh blood cells the electrical charges are very fixed and characteristic, as shown by the rapid and uniform movement of the cells in strong electric fields. The charge of bacteria, however, invariably reverses when the culture is kept for several days.* The negative chemiotaxis described by Bordet† would then have to be interpreted by some such reversal occurring normally in the infected animal, on account of changes in the blood serum or peritoneal fluid.

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* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 641.

† J. Bordet, "Studies on the Serum of Vaccinated Animals," 'Annales de la Société Royale des Sciences médicales et nat. de Bruxelles,' 1895, vol. 4.

## DESCRIPTION OF PLATES.

### PLATE 7.

Fig. 7.—*B. anthracis.* 20 minutes' exposure.
Fig. 8.—*B. anthracis.* 30 minutes' exposure.

### PLATE 8.

Fig. 9.—*B. anthracis.* 50 minutes' exposure.
Fig. 10.—*Pneumococcus.* 70 minutes' exposure.

### PLATE 9.

Fig. 11.—*B. coli communis.* $1\frac{1}{4}$, $4\frac{1}{2}$, and 18 hours' exposure.
Fig. 12.—*B. typhosus.* 1 hour and $2\frac{1}{4}$ hours' exposure.

### PLATE 10.

Fig. 13.—*B. coli communis.* $\frac{1}{2}$ hour and 1 hour's exposure.
Fig. 14.—*B. asiaticæ choleræ.* $\frac{1}{4}$ hour and $\frac{1}{2}$ hour's exposure.
Fig. 15.—*B. dysenterica Shiga.* $\frac{1}{2}$ hour and 1 hour's exposure.

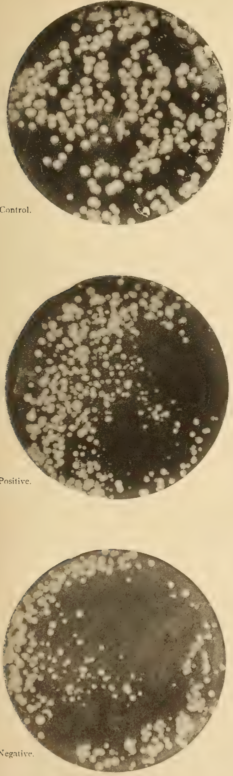
### PLATE 11.

Fig. 16.—*B. asiaticæ choleræ* in hydrogen. 1 hour's exposure.
Fig. 17.—*B. typhosus* in hydrogen. 1 hour's exposure.
Fig. 18.—*B. coli communis* in hydrogen. 1 hour's exposure.

### PLATE 12.

Figs. 3, 4, 5.—Point discharge.
Fig. 6.—Streamers in positive point discharge.
Fig. 20.—*B. typhosus* in nitrogen. Top.
Fig. 21.—*B. asiaticæ choleræ* under quartz and paper. Bottom.

FIG. 7.
B. Anthracis, 20 mins.

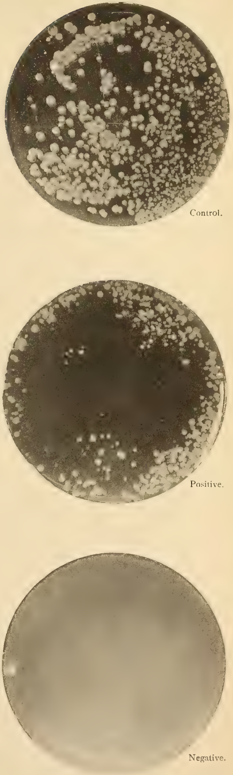


Control.

Positive.

Negative.

FIG. 8.
B. Anthracis, 30 mins.

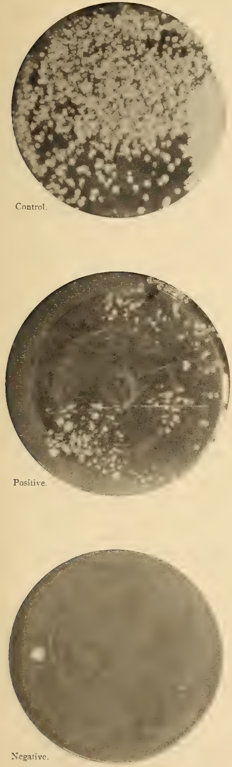


Control.

Positive.

Negative.

FIG. 9.
B. Anthracis, 50 mins.

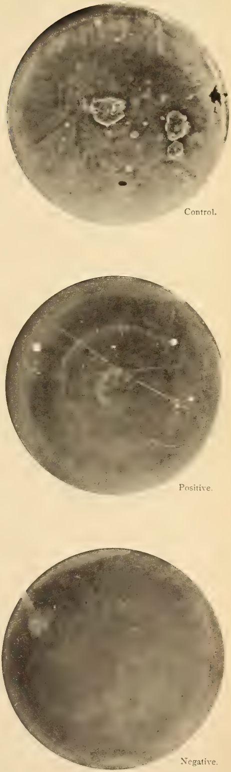


Control.

Positive.

Negative.

FIG. 10.
Pneumococcus. 70 mins.

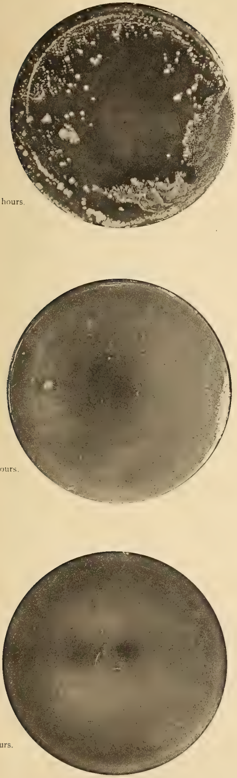


Control.

Positive.

Negative.

FIG. 11.
B. Coli Communis.

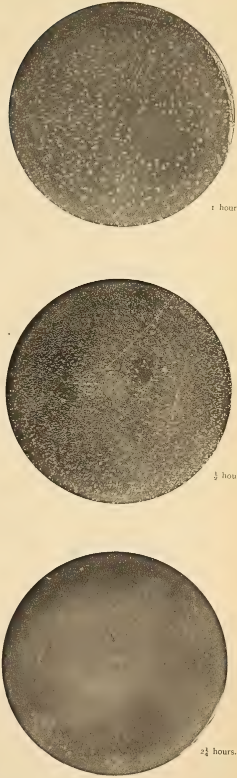


1¼ hours.

4½ hours.

18 hours.

FIG. 12.
B. Typhosus.

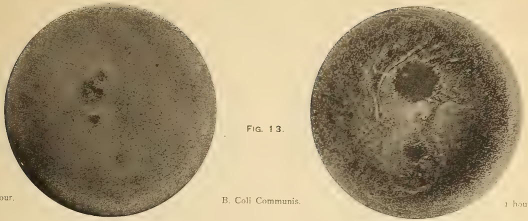


1 hour.

½ hour.

2¾ hours.

Exposed as in Plate 8.

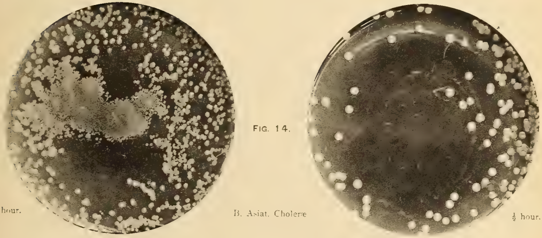


½ hour.

FIG. 13.

B. Coli Communis.

1 hour.

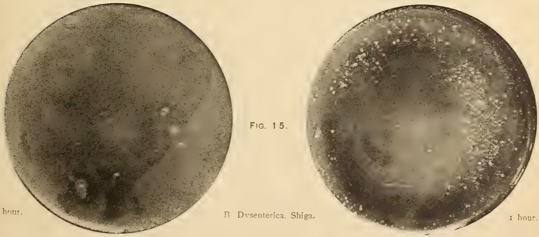


¼ hour.

FIG. 14.

B. Asiat. Choleræ

½ hour.



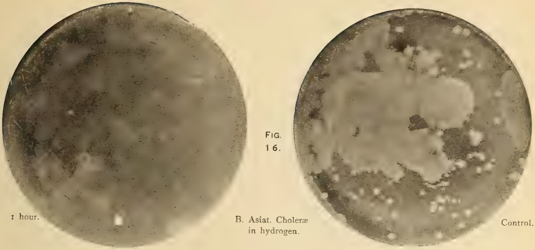
½ hour.

FIG. 15.

B. Dysenterica. Shiga.

1 hour.

FIG. 16.

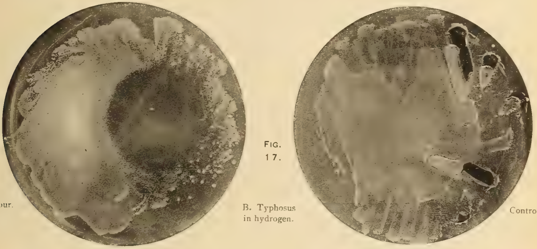


1 hour.

Control.

B. Asiat. Choleræ
in hydrogen.

FIG. 17.

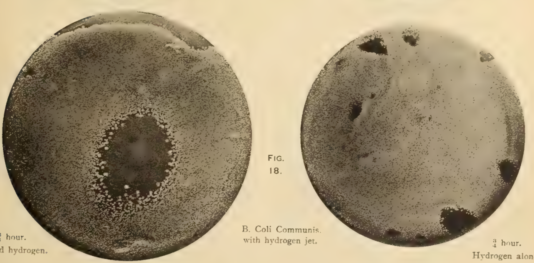


1 hour.

Control.

B. Typhosus
in hydrogen.

FIG. 18.



$\frac{3}{4}$ hour.
Ionised hydrogen.

$\frac{3}{4}$ hour.
Hydrogen alone,
no effect.

B. Coli Communis.
with hydrogen jet.

B. Typhosus in nitrogen.

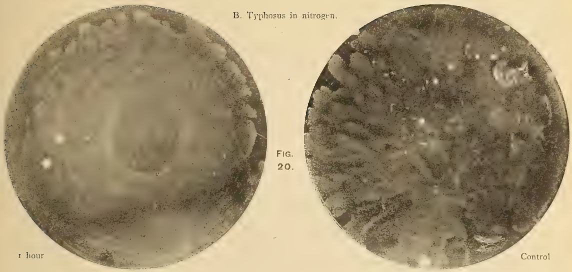


FIG. 20.

1 hour

Control

B. Asiat. Choleræ

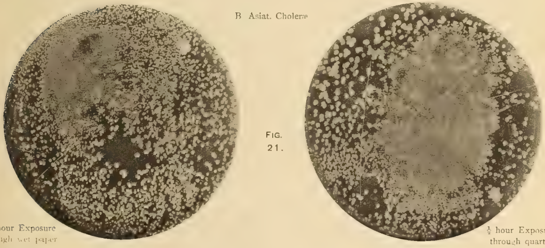


FIG. 21.

¼ hour Exposure through wet paper

½ hour Exposure through quartz.

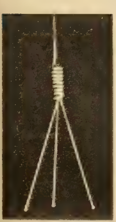


FIG. 3.

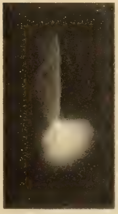


FIG. 4.

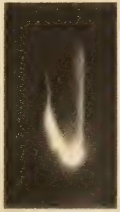


FIG. 5.

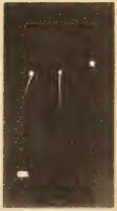


FIG. 6.

# *The Permeability of the Yeast-Cell.*

By Sydney G. Paine, Research Scholar in the Biochemical Department, Lister Institute, London.

(Communicated by Arthur Harden, F.R.S. Received July 12, 1911.)

The question of the permeability of plant membranes and of the protoplasm lining plant cells has received just attention from time to time, the method usually employed being based upon plasmolysis, a phenomenon first described by Nägeli in 1855 (1), and subsequently investigated by Pfeffer (2), De Vries (3), and Overton (4). The results of their experiments tend to show that purely physical diffusion laws cannot always interpret osmotic phenomena as exhibited by living plant cells, but that in some cases there is evidence of specific permeability.

Nathanson (6) finds that the permeability of protoplasm for any substance is not constant, but varies according to the concentration within and without the cells, and he holds that these variations cannot be accounted for in a purely physical manner.

In 1899 Overton (5), in a series of very comprehensive investigations, observed the similarity existing between solutions in oils and in the ectoplasmic layer of the cytoplasm; he showed especially that many substances could be made to enter the plasma by dissolving them in oils, and suggested the hypothesis that the absorption of such substances by living plants might be due to the presence of lecithin and cholesterol in the plasmatic layer. This hypothesis, however, does not account for the semi-permeability exhibited by various plant membranes towards inorganic salts. Again Adrian Brown (7) has shown that the seed coat of *Hordeum vulgare* exhibits a remarkable degree of impenetrability to strong acids and to metallic salts, while it admits of ready diffusion of such substances as alcohol, aldehyde, acetone, iodine, and certain salts of mercury and cadmium. Armstrong (8) has attempted to explain these results on the theory of "hormones," but it appears to the author that strong support to Overton's hypothesis is afforded by these experiments of Adrian Brown. It has been shown by Overton that iodine and mercuric chloride, as well as the above-mentioned organic substances, are readily soluble in cholesterol. Since these include most of the substances which were found by Brown to be capable of entering the barley grain, it seemed advisable to ascertain whether the remaining substances found by him to enter the seed, namely, cadmium iodide and trichloracetic acid, also possessed this property of solubility in oils. Of these, cadmium iodide is not soluble in oil or cholesterol, but is known to form a double compound with lecithin; trichloracetic acid was found to dissolve with great readiness in both xylene and cholesterol. It would, however, be unfair to assume from these facts that the presence or absence of cholesterol and lecithin in the cells is the determining factor for the diffusion of these substances. It seems to the author more likely that there may be some characteristic of the molecule, or possibly a determinate size of particle, which gives to certain substances the property of solubility both in oils and in living protoplasm.

Diffusion must play a very important part in the technique of cytological investigation, since proper fixation depends largely upon the rapid and uniform diffusion of such agents as mercuric chloride, iodine, osmic and chromic acids, etc. Variations in diffusion capacity may possibly account in large measure for the differences observable under the influence of the several fixing agents. It is conceivable, for instance, that in nuclear investigations the extreme sharpness of definition with which the astral rays and spindle-fibres have been brought out by some workers may be due to inferior fixation; in other words, to a contraction along lines of dynamic activity, wherein the protoplasm has become so altered as to possess a coefficient of penetrability for the special fixing agent employed which is different from that of the surrounding medium.

The primary object of this research has been to investigate the osmotic behaviour of the yeast-cell towards those substances which have been found to influence alcoholic fermentation. Harden and Young (9) have shown that when phosphates, arsenites or arsenates are added to yeast-juice and sugar a considerable increase of the rate of fermentation is produced. When, however, these substances are applied to living yeast it has been found that in almost all cases no such result occurs. Slator (10) has shown that when neutral potassium phosphate is added to living yeast the only effect produced on the initial rate is that of a small inhibition. Iwanoff (11) on the contrary, and more recently Euler and Lundeqvist (17), have demonstrated a small increase in the total fermentation produced by addition of phosphates to living yeast and glucose, but these effects are not comparable with that produced on yeast-juice.

Another phenomenon of a similar character is observed in the case of hexosephosphates, which are freely hydrolysed and fermented by yeast-juice, but are scarcely affected by living yeast. As the formation and decomposition of hexosephosphates plays an essential part in alcoholic fermentation by yeast-juice, it is a matter of great importance to ascertain whether these salts can penetrate the cell.

### *The Plasmolysis of Yeast by Various Substances.*

Preliminary experiments were first made on the degree of plasmolysis of yeast-cells produced by immersion of the cells in solutions of different substances. For this purpose equal weights of pressed brewer's yeast were intimately mixed with equal volumes of the several solutions and allowed to stand for varying intervals of time. Well-mixed samples were then drawn up into capillary tubes of 10 cm. length, which were afterwards sealed at one end and spun simultaneously in a centrifuge. The columns of residue and of the clear liquid were then measured in millimetres, and from these the ratio of the length of the column of residue to that of the whole column (residue + liquid) was calculated.

In all these experiments wort and 7 per cent. alcohol were employed as standards, the effect produced being practically the same.

Table I.

| No. | Solution. | Percentage length of column of residue after— | | |
|---|---|---|---|---|
| | | 2 hrs. | 20 hrs. | 70 hrs. |
| 22*a* | Wort | 63·7 | 63·5 | 63·5 |
| *b* | Water | 70·0 | 62·2 | 63·4 |
| *c* | Alcohol, 7 per cent. | 60·5 | 63·3 | 63·4 |
| *d* | Sodium chloride, 0·3 molar | 59·9 | 55·2 | 54·0 |
| *e* | „ 0·1 „ | 66·2 | 61·5 | 61·0 |
| *f* | Sodium phosphate, 0·3 molar | 63·3 | 57·1 | 54·9 |
| *g* | „ 0·1 „ | 70·6 | 63·3 | 62·5 |

It was found that when yeast was treated with water the cells at first increased in volume, but later returned to their original state. An initial dilatation also occurred with decimolar solutions of sodium chloride and sodium phosphate, but eventually, in both these cases, a slight amount of plasmolysis was noted. With 0·3 molar concentrations of these substances no increase in volume was observed, and a considerably greater final degree of plasmolysis was produced than was the case with the weaker solutions.

These numbers show, further, that equilibrium is practically established in 20 hours at air temperature, but not in 2 hours.

Adrian Brown (*loc. cit.*) finds that solutions of certain non-electrolytes seem to possess the power of entering the barley grain, whilst others, such as sugar and urea, do not; also that trichloracetic acid, an acid which becomes strongly ionised in dilute solution, enters quite freely. The fact that most of the entering substances are non-electrolytes, he observes, cannot be taken as an explanation of the diffusion phenomena. A possible solution of the problem has already been advanced (p. 289).

It seemed desirable to ascertain whether these substances would act in a similar manner towards the yeast-cell, it being at first thought that permanent plasmolysis of the cell might be taken as an indication that no diffusion of the dissolved substance into the cell had occurred, an idea which further experiments proved to be untenable (p. 294). The following table contains the results of experiments with acetone, urea, mercuric chloride, cadmium iodide, sulphuric and trichloracetic acids. Since the volumes measured in the narrow tubes were very small, these experiments were made on a larger scale; 50 grm. of pressed yeast were stirred up with 50 c.c. of solutions of the various substances which Adrian Brown found to be of interest. They were allowed to stand for varying lengths of time in the cold room at a temperature ranging from $-2°$ to $+2°$, and were then all spun simultaneously in the centrifuge and the columns of residue and liquid carefully measured. The corresponding volumes were ascertained by gauging the capacity of the vessel.

Table II.

| No. | Solute. | Percentage volume of spun residue after— | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hr. | 2 hrs. | 3 hrs. | 4 hrs. | 20 hrs. | 25 hrs. |
| 176*a* | Sulphuric acid, molar | 43·0 | 41·0 | 36·5 | 37·0 | 36·5 | 36·5 |
| *b* | Sodium chloride, molar | 43·0 | 43·0 | 45·0 | 43·0 | 40·0 | 40·0 |
| *c* | Trichloracetic acid, molar | 42·0 | 42·0 | 37·5 | 37·5 | 37·5 | 37·5 |
| *d* | Alcohol, 7 p c. (control) | 62·0 | 62·0 | 58·0 | 58·0 | 62·0 | 60·0 |
| *e* | Acetone, molar | 64·0 | 62·0 | 60·0 | 60·0 | 64·0 | 62·0 |
| *f* | Urea, molar | 53·0 | 57·0 | 58·0 | 58·0 | 61·0 | 61·0 |
| *g* | Cadmium iodide, molar | 43·0 | 43·0 | 42·0 | 42·0 | 41·0 | 41·0 |
| *h* | Mercuric chloride (satd.) | 54·5 | 50·5 | 50·5 | 43·0 | 38·5 | 35·5 |

These results exhibit striking differences when compared with Adrian Brown's experiments. When this observer immersed dried grains of barley in different solutions, water entered as freely and rapidly from solutions of alcohol, acetone, and trichloracetic acid as from pure water, a fact which was interpreted as showing that these substances readily penetrated through the diffusion membrane. In *d* and *e* above alcohol of 7 per cent. and acetone produced no permanent plasmolysis and would seem to diffuse quite readily. Urea also produced no permanent plasmolysis, in striking contrast to Brown's result, where the entrance of water was strongly inhibited. The behaviour of trichloracetic acid also stands in contrast to its behaviour towards the barley grain.

In the experiments with cadmium iodide and mercuric chloride considerable plasmolysis occurred, but this fact cannot be taken to indicate that no diffusion of these substances had taken place, since a marked change was observed in the appearance of the yeast. The cells became much paler in colour and more opaque, while the liquid assumed a dark brownish-grey colour. From the solid appearance of the cells, it would seem that these salts had penetrated through the membrane and coagulated and contracted the cytoplasm; this appears the more evident in the case of the relatively weaker solution of mercuric chloride, where plasmolysis went on slowly up to the end of three hours, after which a strong and rapid contraction took place. These facts are explicable on the assumption that the proteins of the ectoplasm are slowly coagulated during the first three hours and by contraction leave open access for the solution to the inner layers.

In another series of experiments 10 grm. of pressed yeast were weighed out into each of several Nessler glasses and treated with 20 c.c. of the solutions tabulated below. The tubes were allowed to stand in ice water during about 20 hours. They were then centrifuged in batches of four, each batch being spun for exactly 21 minutes. The tubes were then weighed, the liquids poured off into measuring vessels and the weights of the residues ascertained by re-weighing the tubes.

No plasmolysis was produced by solutions of acetone, urea and the lower concentrations of alcohol up to 10 per cent. With the higher concentrations

Table III.

| No. | Solute. | Total weight. | Weight of residue. | Percentage of residue. | Volume of liquid poured off. |
|---|---|---|---|---|---|
| 178, 1 | Water | 29·8 | 14·3 | 48·0 | 15·5 |
| 2 | Alcohol, 7 per cent | 29·5 | 14·0 | 47·5 | 15·6 |
| 3 | ,, 10 ,, | 29·3 | 13·9 | 47·1 | 15·5 |
| 4 | ,, 20 ,, | 29·1 | 13·0 | 44·7 | 16·5 |
| 5 | ,, 25 ,, | 29·0 | 12·6 | 43·4 | 17·2 |
| 6 | ,, 30 ,, | 28·7 | 11·2 | 39·0 | 18·3 |
| 7 | Acetone, molar | 29·9 | 14·7 | 49·2 | 15·0 |
| 8 | Urea, molar | 29·9 | 14·3 | 47·8 | 15·5 |
| 9 | Glycerine, molar | 29·8 | 12·9 | 43·3 | 16·5 |
| 10 | ,, $\frac{1}{10}$ molar | 29·7 | 14·6 | 49·1 | 15·5 |
| 11 | Sulphuric acid, $\frac{1}{2}$ molar | 30·4 | 8·7 | 28·6 | 21·7 |
| 12 | Acetic acid, molar | 29·8 | 8·6 | 28·9 | 21·0 |
| 13 | Sodium chloride, molar | 30·5 | 10·5 | 34·4 | 19·0 |
| 14 | ,, $\frac{1}{10}$ molar | 29·9 | 14·0 | 46·8 | 15·8 |
| 15 | Sodium acetate, molar | 30·4 | 11·7 | 38·5 | 18·0 |
| 16 | Sodium sulphate, $\frac{1}{2}$ molar | 30·9 | 12·1 | 39·1 | 18·0 |
| 17 | Magnesium sulphate, molar | 31·5 | 11·8 | 37·5 | 18·5 |
| 18 | ,, $\frac{1}{10}$ molar | 30·9 | 14·6 | 47·3 | 15·0 |
| 19 | Sodium phosphate, $\frac{3}{10}$ molar | 30·4 | 12·8 | 42·1 | 17·4 |
| 20 | Sodium arsenate, $\frac{2}{10}$ molar | 30·4 | 12·7 | 41·8 | 17·5 |

of the latter plasmolysis was well marked, the effect increasing with increasing concentration. Since no appreciable effect is produced by concentrations up to 10 per cent. it seems possible that diffusion of alcohol is freely permitted. The plasmolysis produced by more concentrated alcohol may be a result of changes in the molecular constitution of the protoplasm. Comparison with the case of mercuric chloride tends to strengthen this view.

The liability to plasmolysis by 20 per cent. alcohol exhibited by different samples of yeast seems to vary with the physical condition of the yeast.

In the experiments given in Table III 10 grm. of pressed yeast were stirred up with 20 c.c. solution, allowed to stand over night in the cold room, and centrifuged next morning.

Table IV.

| Solution. | Yeast A. | | Yeast B. | | Yeast C. | | Yeast D. | | Yeast E. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Volume of liquid poured off. | Weight of residue. | Volume of liquid poured off. | Weight of residue. | Volume of liquid poured off. | Weight of residue. | Volume of liquid poured off. | Weight of residue. | Volume of liquid poured off. | Weight of residue. |
| Water .................. | 14·0 | — | 15·0 | 14·8 | 15·5 | 14·0 | 13·7 | 16·1 | 15·5 | 14·3 |
| Alcohol, 5 per cent.... | — | — | 14·5 | 14·7 | 16·0 | 14·1 | | | | |
| „ 7 „ ... | 14·0 | — | 15·0 | 14·6 | 14·5 | 13·9 | 14·1 | 15·7 | 15·6 | 14·0 |
| „ 10 „ ... | — | — | 14·5 | 14·5 | 15·0 | 14·0 | 14·0 | 15·7 | 15·5 | 13·9 |
| „ 15 „ ... | 15·0 | — | 14·0 | 14·5 | 15·0 | 14·1 | | | | |
| „ 20 „ ... | 17·0 | — | 15·5 | 14·0 | 15·5 | 14·4 | 15·0 | 13·8 | 16·5 | 13·0 |
| „ 25 „ ... | — | — | — | — | — | — | 16·2 | 13·1 | 17·2 | 12·6 |
| „ 30 „ ... | — | — | — | — | — | — | 21·0 | 9·4 | 18·3 | 11·2 |

Yeast C was an old sample which had been kept in the cold room for about 24 hours after being received from the brewery, and it is worthy of note that no plasmolysis of this yeast by concentrations of alcohol up to 20 per cent. could be detected by this method.

The fact that permanent plasmolysis of yeast is produced by higher concentrations of alcohol, by mercuric and cadmium salts which precipitate the proteins within the cell, and by acids which prevent the activity of the cell, shows that for this cell the existence of permanent plasmolysis is no criterion of the non-diffusibility of the solutions producing it.

In order to arrive at definite results on this subject, it was clearly seen that quantitative estimations of the substance under investigation in the yeast-cells and in the liquid surrounding the cells would be necessary. Attempts were therefore made to obtain the yeast-cells minus the liquid which normally fills the interstices in an ordinary cake of yeast.

This was eventually accomplished by enclosing the moist yeast cake of the brewery, or yeast obtained as residue after centrifuging, in chain cloth and subjecting it to the pressure of a small hand press. A white friable cake of yeast was obtained which appeared to be composed of dry cells. This was proved to be the actual fact by the following series of experiments:—(1) Total solid estimations in the same pressed cake gave uniform results, showing the cake to be homogeneous. (2) Two pressings of the same yeast-paste gave dry pressed cakes with the same total solid content. (3) Samples of brewery yeast were pressed out and subsequently suspended in the expressed wort, centrifuged and again pressed out, the total solids in the two press cakes were exactly equal. (4) Direct estimations of a salt solution left in the interstices of press cake showed that the greatest volume of liquid thus held by 100 grm. of dry pressed yeast was 0·5 c.c.

### *Method of Experiment.*

The yeast was prepared by pressing out the cake of yeast as received from the press of the brewery, washing being avoided in order to prevent disturbance of the equilibrium of the cell contents. A considerable amount of wort was thus removed. A known weight of this dry yeast was then suspended in a certain volume of the liquid under experiment and allowed to stand for about 20 hours in the cold, after which it was found that osmotic equilibrium between the cells and the solution was attained. The mixture was then centrifuged until the liquid portion was cleared from suspended yeast-cells. The clear fluid was then poured off and the pasty yeast residue was pressed out as described above.

In order to ascertain the weights of yeast-cells and liquid after the experiment, and the distribution of the solute under examination, the following determinations were necessary:—Total solid estimations of the initial and final liquid, and of the initial and final pressed yeast, together with estimations of the dissolved substance in the initial and final liquid, and, in all cases where this was possible, in the initial and final yeast.

The assumption has been made that the total solid matter present in the mixture remains constant during the experiment, an assumption only justified when no loss of carbon dioxide owing to auto-fermentation of the yeast takes place.

### *Effect of Auto-fermentation.*

A small loss of carbon dioxide, accompanied by production of alcohol, does always take place, and the results are subject to error arising from this cause. It was found by direct experiment that, under the conditions employed, a maximum loss of about 0·9 grm. of solid was caused by auto-fermentation.

A loss of 1 grm. in total solids during an experiment has been found to produce a positive error of 5 per cent. on the calculated weight of liquid outside the yeast-cells, so that the results to be given later must be considered to be liable to an error of this order. To eliminate this factor as far as possible, the mixture was allowed to stand in the cold room at a temperature ranging from $-2$ to $+1°$.

### *Calculation of the Formula for Obtaining the Weight of Liquid outside the Yeast-cells.*

Let $l$ = weight of initial liquid, $y$ = weight of initial yeast, then the total weight $W = l + y$; and if $g$ = percentage of solids in $l$, and $e$ = percentage of solids in $y$, then the total solid matter present $V = \frac{lg}{100} + \frac{ye}{100}$.

Both these values W and V are assumed to remain constant during the experiment.

Further, let L = weight of final liquid, Y = weight of final yeast, then $Y = W - L$; and if G = percentage of solids in L, and E = percentage of solids in Y, then the total solid matter $V = \frac{LG}{100} + \frac{YE}{100}$. Substituting for Y in terms of W and L,

$$\frac{LG}{100} + \frac{(W-L)E}{100} = V,$$

whence $L = \frac{EW - 100V}{E - G}$, and Y is obtained by difference from W.

Having thus calculated the weights of liquid and yeast at the end of the experiment, the distribution of the substance under investigation, before and after treatment, is found from the analyses of the initial and final liquid and the initial and final yeast; at the same time, any interchange of other solid matter and of water is made manifest.

### *Alcohol.*

Table V shows the results of experiments with alcohol of various concentrations. This substance was chosen as it might be expected to diffuse freely through the envelope of the cell. Assuming the whole of the water within the cells to be available for mixture with alcohol, the concentrations of alcohol inside and outside the cells, after osmotic equilibrium had been established, would be equal.

Now the grammes of substance (in this case alcohol) per 100 grm. of water within the yeast (P), divided by the grammes of substance per 100 grm. of water outside the cells ($P_1$), gives a measure (K) of the amount of diffusion which has taken place. In the case under discussion, therefore, K would be expected to be equal to unity.

In these experiments the increase of alcohol due to auto-fermentation could not be neglected. The total amount of alcohol present at the end of the experiment was therefore determined by analysis of the liquid poured off and of the residue after centrifuging. The weight of alcohol due to auto-fermentation was thus found by difference from the original amount, and was embodied in the calculation for the weight of liquid outside the cells. The formation of an amount of alcohol (F) during an experiment occasions a loss of an approximately equal amount of carbon dioxide to be

Table V.—Diffusion of Alcohol of varying Concentrations.

| No. | Conditions. | | Yeast. | | Liquid. | | P. | $P_1$. | K. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial. | Final. | Initial. | Final. | | | |
| 75 | Alcohol, 2 ·5 molar, stood 3 hrs. at room temperature | Total ... | 100 ·00 | 95 ·70 | 98 ·00 | 101 ·90 | | | |
| | | Alcohol | 4 ·29 | 5 ·80 | 11 ·47 | 10 ·78 | 9 ·91 | 12 ·08 | 0 ·82 |
| 76 | „ | Total ... | 100 ·00 | 95 ·50 | 98 ·00 | 102 ·30 | | | |
| | | Alcohol | 4 ·50 | 5 ·60 | 11 ·47 | 10 ·42 | 9 ·13 | 10 ·95 | 0 ·83 |
| 77 | Alcohol, 2 ·5 molar, stood 20 hrs. in cold room | Total ... | 100 ·00 | 98 ·17 | 98 ·00 | 99 ·57 | | | |
| | | Alcohol | 3 ·14 | 5 ·50 | 11 ·47 | 9 ·50 | 8 ·97 | 10 ·65 | 0 ·84 |
| 80 | „ | Total ... | 100 ·00 | 91 ·08 | 98 ·00 | 106 ·16 | | | |
| | | Alcohol | 3 ·48 | 5 ·36 | 11 ·47 | 10 ·62 | 9 ·62 | 11 ·30 | 0 ·85 |
| 100 | Alcohol, 1 ·3 molar, stood 20 hrs. in cold room | Total ... | 100 ·00 | 97 ·56 | 98 ·80 | 100 ·47 | | | |
| | | Alcohol | 3 ·39 | 3 ·42 | 6 ·00 | 6 ·63 | 5 ·31 | 7 ·13 | 0 ·74 |
| 101 | „ | Total ... | 100 ·00 | 94 ·01 | 98 ·80 | 104 ·31 | | | |
| | | Alcohol | 3 ·47 | 3 ·55 | 6 ·00 | 6 ·84 | 5 ·95 | 7 ·07 | 0 ·84 |
| 81 | Water, stood 20 hrs. in cold room | Total ... | 50 ·00 | 47 ·07 | 100 ·00 | 102 ·59 | | | |
| | | Alcohol | 2 ·39 | 0 ·64 | — | 2 ·11 | 1 ·96 | 2 ·11 | 0 ·92 |
| 85 | „ | Total ... | 100 ·00 | 94 ·16 | 100 ·00 | 105 ·48 | | | |
| | | Alcohol | 4 ·44 | 1 ·37 | — | 3 ·42 | 2 ·16 | 3 ·38 | 0 ·64 |
| 90 | „ | Total ... | 100 ·00 | 99 ·83 | 100 ·00 | 99 ·90 | | | |
| | | Alcohol | 4 ·65 | 1 ·86 | — | 3 ·19 | 2 ·66 | 3 ·34 | 0 ·79 |

subtracted from the total weight (W), and a loss of approximately twice the amount of solid matter (2F) to be subtracted from V, since the alcohol is formed according to the equation

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH.$$

If F = weight of alcohol by auto-fermentation, W−F = the total weight at the end of the experiment, and V−2F = the weight of solid matter finally present, and the formula given on p. 296 becomes

$$L = \frac{E(W-F)-100(V-2F)}{E-G} = \frac{EW-100V-F(E-200)}{E-G},$$

and Y is obtained by difference from W−F.

The table shows very clearly that alcohol penetrates freely through the cytoplasm of yeast, but the interesting fact is observed that when equilibrium is established the ratio of alcohol to water is, in every case, less within the cell (P) than it is outside ($P_1$), and that these ratios stand to one another in a fairly constant proportion (K).

This points to the possibility that some of the water of the protoplasm is bound up in such a manner as to render it unavailable as a solvent for alcohol. This view is supported by the high value for K found in Experiment 81, wherein old yeast was employed which contained a very large vacuolar space and a correspondingly decreased layer of cytoplasm.

The method is specially interesting, as it affords a very clear insight into the interchange of material occurring between the cells and the surrounding liquid. For instance, in Experiment 81 (yeast in water), 0·36 grm. of alcohol have been formed by auto-fermentation within the yeast, bringing the total alcohol up to 2·75 grm. Of this 2·11 grm. have passed out into the surrounding water, leaving 0·64 grm. in the final yeast; 0·70 grm. of solid matter have passed out from the yeast, and 0·73 grm. of solids have been fermented. At the same time there has been an entrance of 0·21 grm. of water into the cells, which is also accounted for as having left the liquid.

Since in these experiments the value of K appeared to be independent of the concentration of the alcohol, it seemed advisable to investigate this further, and also to try the effect of variations in other directions. Since the factor K depends solely upon the analyses of the components of the final system, in each of the experiments about to be described only two estimations of total solids and two of alcohol were necessary.

The results of these experiments are contained in the following Table VI. In all cases, except where otherwise stated, the duration of the diffusion was 20 hours at the temperature of the cold room :—

Table VI.—Shewing Diffusion of Alcohol.

| No. | Alcohol. | Time of standing. | Yeast. | | | Liquid. | | | P. | P$_1$. | K. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Solids. | Alcohol. | Water. | Solids. | Alcohol. | Water. | | | |
| | per cent. | hrs. | per cent. | per cent. | per cent. | per cent. | per cent. | per cent. | | | |
| Effect of varying Concentration. | | | | | | | | | | | |
| 119 | 20 | 20 | 38·63 | 8·15 | 53·22 | 1·52 | 15·02 | 83·46 | 15·31 | 17·99 | 0·85 |
| 120 | 10 | 20 | 27·95 | 5·97 | 66·08 | 0·83 | 9·33 | 89·84 | 9·04 | 10·38 | 0·87 |
| 121 | 5 | 20 | 30·49 | 3·48 | 66·03 | 0·91 | 5·75 | 93·34 | 5·27 | 6·16 | 0·85 |
| 122 | 0 | 20 | 28·71 | 1·72 | 69·57 | 0·92 | 2·94 | 96·14 | 2·47 | 2·98 | 0·83 |
| Effect of varying Time of Standing. | | | | | | | | | | | |
| 131 | 20 | 17 | 33·00 | 7·67 | 59·33 | 0·69 | 13·67 | 85·64 | 12·93 | 15·96 | 0·81 |
| 132 | 20 | 41 | 34·49 | 8·14 | 57·37 | 0·74 | 13·90 | 85·36 | 14·19 | 16·28 | 0·87 |
| 133 | 20 | 65 | 34·99 | 7·74 | 57·27 | 1·03 | 13·80 | 85·17 | 13·52 | 16·20 | 0·83 |
| 134 | 20 | 89 | 33·46 | 9·88 | 56·66 | 1·32 | 13·87 | 84·81 | 13·85 | 16·35 | 0·85 |
| In Auto-fermented Yeast. | | | | | | | | | | | |
| 81 | 0 | 20 | 29·26 | 1·37 | 69·37 | 0·69 | 2·06 | 97·25 | 1·96 | 2·11 | 0·92 |
| 139 | 10 | 20 | 30·82 | 4·87 | 64·31 | 1·29 | 8·27 | 90·44 | 7·57 | 9·14 | 0·83 |
| 144 | 10 | 20 | 30·76 | 6·06 | 63·18 | 4·07 | 9·40 | 86·53 | 9·59 | 10·86 | 0·88 |
| Effect of varying Quantity of Liquid. | | | | | | | | | | | |
| | Yeast. | Alcohol, 10 per cent. | | | | | | | | | |
| 141 | 50 grm. | 50 cc. | 34·50 | 4·11 | 61·39 | 0·95 | 7·73 | 91·32 | 6·69 | 8·46 | 0·79 |
| 142 | 50 ,, | 100 ,, | 34·61 | 4·35 | 61·04 | 0·47 | 7·86 | 91·67 | 7·13 | 8·57 | 0·83 |
| 143 | 50 ,, | 200 ,, | 34·88 | 4·10 | 61·02 | 0·27 | 8·05 | 91·68 | 6·72 | 8·78 | 0·76 |

In this table, the factor K is found to be remarkably uniform, and to be uninfluenced by variations in the conditions. It is further noteworthy that, under the action of concentrations ranging from 0 to 10 per cent. (Experiments 119–122), no marked variation in the solid content of the yeast is produced. This stands in further confirmation of the results obtained during the earlier experiments on plasmolysis given in Table III, p. 293, in which it is shown that the plasmolysing effect of alcohol is inappreciable until a concentration of 20 per cent. is employed. Again, as was previously observed, the extent of plasmolysis by the higher concentration is not uniform in different samples of yeast; for instance, in the yeast of Experiment 119, an increase of 10 per cent. in the total solids is observed, while in the yeast of similar experiments the increase was only 1·8 and 4·7 per cent. It was noticed also that, with this concentration, a change had taken place within the cell, making it impossible to obtain the usual white friable press cake, but rather a brown-coloured and putty-like mass.

This change may possibly take the form of a contraction of the cytoplasm of the cell, and, since the depth of the layer of cytoplasm varies under different conditions of the yeast, the amount of plasmolysis sustained by a given sample of yeast when immersed in 20 per cent. alcohol may possibly be determined by the relative proportions of the cell occupied by the cytoplasm and by the vacuole.

In Experiments 131–134 a gradual diffusion of solid matter from the yeast into the surrounding liquid is observed to have taken place; this is probably due to the production of diffusible solids by autolysis of the cell contents.

In Experiments 81 and 139 yeast was employed which had been kept in the cold room for three days. The yeast of 144 was a sample which had been dried in the air at room temperature. The original percentage of total solids in this dry yeast was 68·4 per cent. On immersion in 10 per cent. alcohol the yeast absorbed liquid very quickly, and eventually, after 20 hours, 50 grm. had increased to about 110 grm. Notwithstanding this large influx of liquid, the diffusion factor is seen to be not widely different from the normal.

### *Sodium Chloride.*

This substance was taken as being a typical salt dissociated into its ions more or less completely in dilute solution. Four experiments were made, the results of which are given below. The salt was estimated in the liquid by the method of Carius, the amount in the yeast being calculated by difference. Preliminary experiments had shown that when 50 grm. of pressed yeast

Table VII.—Diffusion of M/10 Sodium Chloride.

| No. | Conditions. | | Liquid. | | Yeast. | | P. | $P_1$. | K. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial. | Final. | Initial. | Final. | | | |
| 60 | Allowed to stand 20 hrs. in cold store | Total ... | 100·50 | 101·00 | 50·00 | 49·50 | | | |
| | | NaCl ... | 0·58 | 0·54 | nil | 0·04 | 0·11 | 0·54 | 0·21 |
| 61 | Allowed to stand 20 hrs. in cold store | Total ... | 100·50 | 100·30 | 50·00 | 50·20 | | | |
| | | NaCl ... | 0·58 | 0·55 | nil | 0·03 | 0·08 | 0·55 | 0·15 |
| 64 | Allowed to stand 3 hrs. at room temperature | Total ... | 100·50 | 104·30 | 50·00 | 46·20 | | | |
| | | NaCl ... | 0·58 | 0·58 | nil | nil | — | 0·56 | — |
| 65 | Allowed to stand 3 hrs. at room temperature | Total ... | 100·50 | 101·60 | 50·00 | 48·90 | | | |
| | | NaCl ... | 0·58 | 0·58 | nil | nil | — | 0·58 | — |

were suspended in distilled water and the mixture centrifuged, only a faint trace of milkiness was produced by the addition of acid silver nitrate to the liquid poured off.

These results show that the diffusion of sodium chloride is slow. A definite quantity of substance enters the cell when yeast is suspended in M/10 sodium chloride solution and allowed to stand over night, although no diffusion is noticed after a suspension of three hours only.

### *Ammonium Sulphate.*

The next experiments were made with ammonium sulphate, as being a substance which is of service to the yeast, and which is a sufficient source of nitrogen in artificial culture. In these experiments two concentrations of the solution have been employed, of approximately one-tenth and three-tenths molar respectively.

Table VIII.

| No. | Conditions. | | Liquid. | | Yeast. | | P. | $P_1$. | K. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial. | Final. | Initial. | Final. | | | |
| 67 | Stood 20 hrs. in cold store. Solution, 0·1 molar | Total ......<br>$(NH_4)_2SO_4$ | 100·40<br>1·31 | 97·84<br>1·21 | 50·00<br>nil | 52·56<br>0·10 | 0·28 | 1·26 | 0·22 |
| 68 | Stood 20 hrs. in cold store. Solution, 0·1 molar | Total ......<br>$(NH_4)_2SO_4$ | 100·50<br>1·31 | 99·21<br>1·19 | 50·00<br>nil | 51·29<br>0·12 | 0·34 | 1·22 | 0·28 |
| 69 | Stood 3 hrs. in cold store. Solution 0·1 molar | Total ......<br>$(NH_4)_2SO_4$ | 100·60<br>1·29 | 97·30<br>1·23 | 100·00<br>nil | 103·30<br>0·06 | 0·09 | 1·28 | 0·07 |
| 70 | Stood 3 hrs. at room temperature. Solution, 0·1 molar | Total ......<br>$(NH_4)_2SO_4$ | 100·60<br>1·31 | 99·48<br>1·21 | 50·00<br>nil | 51·12<br>0·10 | 0·28 | 1·24 | 0·23 |
| 71 | Stood 3 hrs. at room temperature. Solution, 0·1 molar | Total ......<br>$(NH_4)_2SO_4$ | 100·60<br>1·31 | 102·50<br>1·28 | 50·00<br>nil | 48·10<br>0·03 | 0·09 | 1·27 | 0·07 |
| 145 | Stood 20 hrs. in cold store. Solution, 0·1 molar. Air-dried yeast | Total ......<br>$(NH_4)_2SO_4$ | 90·10<br>1·14 | 58·78<br>0·98 | 30·00<br>nil | 61·32<br>0·16 | 0·37 | 1·76 | 0·22 |
| 149 | Stood 20 hrs. in cold store. Solution, 0·3 molar | Total ......<br>$(NH_4)_2SO_4$ | 50·50<br>1·84 | 58·87<br>1·71 | 50·00<br>nil | 41·63<br>0·13 | 0·47 | 3·00 | 0·15 |
| 150 | Stood 20 hrs. in cold store. Solution, 0·3 molar. Air-dried yeast | Total ......<br>$(NH_4)_2SO_4$ | 100·00<br>3·65 | 90·86<br>3·40 | 50·00<br>nil | 59·14<br>0·25 | 0·61 | 3·91 | 0·16 |
| 151 | Stood 20 hrs. in cold store. Solution, 0·3 molar. Air-dried yeast | Total ......<br>$(NH_4)_2SO_4$ | 100·00<br>3·65 | 75·44<br>3·13 | 50·00<br>nil | 74·56<br>0·52 | 1·03 | 4·41 | 0·23 |

The ammonium sulphate was estimated in the initial and final liquids only, the amount in the final yeast being found by difference.

The results with ammonium sulphate are found to be very similar to those obtained with sodium chloride.

Experiments 145 and 151 are specially interesting as the initial yeast in these cases contained a large percentage of solid matter. In the former, where one-tenth molar ammonium sulphate was employed, 33·17 grm. of water have entered the yeast while only 0·16 grm. of the salt have been carried in, and in the latter from three-tenths molar solution 25·47 grm. of water and 0·52 grm. of salt have entered. The ratio of the concentration inside the cells to the concentration outside is the same in both cases.

The rate at which a sample of air-dried yeast will absorb water and recover turgescence is very remarkable. In No. 145 when 30 grm. of the yeast were mixed with 60 c.c. of solution such a stiff paste was obtained, within two minutes, that it could only be stirred with difficulty. It is further worthy of note that yeast which has been dried in air returns to its normal condition of turgescence when immersed in water, as shown by the percentage of total solids. In all samples of fresh pressed yeast the total solid content has been found to vary from 28 to 35 per cent. Total solid estimations of three air-dried samples gave 68·4, 50·5, and 37·5 per cent.; when immersed in water these yeasts became turgid with a normal solid content of 30·5, 31, and 32·4 per cent. respectively.

The envelope of such dried and shrivelled cells, though readily permeable by water, does not admit of the entrance of a 1/10 molar solution of ammonium sulphate, but selects from it a large quantity of water and only a relatively small quantity of the salt. The liquid surrounding the cells therefore becomes considerably concentrated. In Experiment 145, for instance, the concentrations of salt in the initial and final liquids were 1·26 and 1·66 respectively.

### *Copper Sulphate.*

A peculiar resistance to the entrance of copper salts is exhibited by the protoplasm of *Penicillium glaucum* (12), growth of which has been found possible on a medium containing as much as 21 per cent. copper sulphate, although very much smaller quantities down to 3 per cent. have occasionally proved destructive. In view of this result it seemed advisable to investigate the effect of solutions containing copper upon the protoplasm of the yeast-cell. 100 grm. of yeast were suspended in 100 grm. 1/10 molar $CuSO_4$, allowed to stand for the usual time in the cold room, and the distribution of the copper determined.

Table IX.

| Initial liquid. | | Initial yeast. | | Final liquid. | | Final yeast. | |
|---|---|---|---|---|---|---|---|
| Weight. | $CuSO_4$. | Weight. | $CuSO_4$. | Weight. | $CuSO_4$. | Weight. | $CuSO_4$. |
| grm.<br>100 | grm.<br>0·78 | grm.<br>100 | grm.<br>— | grm.<br>128·4 | grm.<br>0·36 | grm.<br>71·6 | grm.<br>0·43 |

A remarkable degree of plasmolysis was observed and the shrunken cells were of a pale green colour and solid appearance, the cytoplasm had evidently entered into combination with the $CuSO_4$ and had been precipitated thereby, so that the factor K in this case is much greater than unity, namely, 3·36. When this yeast was added to sugar solution no fermentation was produced.

*Sodium Phosphate.*

These experiments have been made with solutions of the di-sodium salt of two concentrations, roughly 1/10 and 3/10 molar, and with water.

Table X.

| No. | Conditions. | | Yeast. | | Liquid. | |
|---|---|---|---|---|---|---|
| | | | Initial. | Final. | Initial. | Final. |
| 103 | Liquid containing 1·42 grm./100 c.c. $Na_2HPO_4$, stood 20 hrs. in cold store | Total ...... | 50·00 | 50·34 | 50·50 | 50·16 |
| | | $P_2O_5$ ...... | 0·75 | 0·76 | 0·35 | 0·38 |
| 104 | „ „ | Total ...... | 50·00 | 49·91 | 50·50 | 50·59 |
| | | $P_2O_5$ ...... | 0·81 | 0·87 | 0·35 | 0·36 |
| 107 | „ „ | Total ...... | 100·00 | 99·90 | 101·30 | 101·40 |
| | | $P_2O_5$ ...... | 1·63 | 1·64 | 0·71 | 0·71 |
| 109 | „ „ | Total ...... | 100·00 | 100·74 | 101·50 | 100·76 |
| | | $P_2O_5$ ...... | 1·39 | 1·55 | 0·71 | 0·63 |
| 111 | Liquid containing 4·16 grm./100 c.c. $Na_2HPO_4$ | Total ...... | 100·00 | 95·50 | 104·00 | 108·50 |
| | | $P_2O_5$ ...... | 1·63 | 1·97 | 2·08 | 1·80 |
| 112 | „ „ | Total ...... | 100·00 | 96·60 | 104·00 | 107·40 |
| | | $P_2O_5$ ...... | 1·59 | 2·01 | 2·08 | 1·80 |
| 105 | Water ........................................ | Total ...... | 100·00 | 102·02 | 100·00 | 97·98 |
| | | $P_2O_5$ ...... | 1·43 | 1·49 | — | trace |
| 108 | Water ........................................ | Total ...... | 50·00 | 53·16 | 50·00 | 46·84 |
| | | $P_2O_5$ ...... | 0·81 | 0·80 | — | 0·01 |
| 114 | Water ........................................ | Total ...... | 100·00 | 107·80 | 100·00 | 92·20 |
| | | $P_2O_5$ ...... | 1·53 | 1·56 | — | trace |

In the estimations, the organic matter was destroyed by Neumann's method and the phosphoric acid was precipitated with magnesium citrate mixture.

With the weaker concentration, which was found to be isotonic with yeast, no exchange of phosphoric acid took place, but from a solution containing 4 per cent. of sodium phosphate, approximately 0·3 molar, entrance of phosphoric acid into the cells was well marked.

### *Sodium Hexosephosphate.*

For the purpose of this research hexosephosphate was of all substances of greatest interest, since Harden and Young (13) have found that hexosephosphoric acid is continually being built up and broken down again in the fermentation of sugar by yeast-juice. When they added this substance to living yeast, however, no evidence of its fermentation could be obtained. It was of special importance, therefore, to determine whether any of the substance had been able to diffuse into the yeast-cells.

The solution of hexosephosphoric acid was prepared by the method described by Young (14) and was neutralised to litmus with caustic soda. Four concentrations of the salt have been employed and the results are given in the following table. In each case the time of standing was 20 hours at a temperature between −2 and 0°.

Table XI.—Diffusion of Sodium Hexosephosphate.

| No. | Conditions. | | Yeast. | | Liquid. | |
|---|---|---|---|---|---|---|
| | | | Initial. | Final. | Initial. | Final. |
| 169 | Concentration, 0·035 molar = 0·14 normal. Standing 20 hrs. in cold room | Total...... | 100·00 | 89·80 | 100·00 | 110·20 |
| | | $P_2O_5$ ...... | 1·42 | 1·36 | 0·50 | 0·51 |
| 174 | Concentration, 0·06 molar = 0·24 normal | Total...... | 100·00 | 102·50 | 100·00 | 97·50 |
| | | $P_2O_5$ ...... | 1·42 | 1·55 | 0·84 | 0·80 |
| 172 | Concentration, 0·126 molar = 0·504 normal | Total...... | 100·00 | 92·90 | 100·00 | 107·10 |
| | | $P_2O_5$ ...... | 1·26 | 1·52 | 1·80 | 1·56 |
| 177 | Concentration, 0·23 molar = 0·93 normal | Total...... | 100·00 | 92·66 | 100·00 | 107·34 |
| | | $P_2O_5$ ...... | 1·66 | 1·99 | 3·33 | 2·98 |

The results are strikingly similar to those obtained with sodium phosphate. Where the concentration was small, as, for instance, in Experiment 169, no definite entrance of phosphorus took place; with higher concentrations, however, the increase of $P_2O_5$ in the yeast became well marked. Experiment 177 showed that 0·33 grm. of $P_2O_5$, equal to 2·5 c.c. of molar solution and to 1/10 of the total amount in the liquid, have been transferred from the liquid to the yeast. The influence of this solution upon the fermentation of yeast was studied according to the method described in a preliminary communication by Harden and Paine (15), and, although the initial rate of auto-fermentation was increased, the total volume of gas yielded was not greater than that given by a water control, and, moreover, the rate of auto-fermentation produced was exactly comparable to the rate under the influence of sodium phosphate of the same normality. It would seem from this that, although this substance is capable of entering the yeast-cell, it is not able to penetrate through to the sphere of activity of the hydrolysing enzyme.

### *Sodium Arsenate.*

Sodium arsenate was specially interesting, since Harden and Young (16) have found that solutions of arsenates have an enhancing influence on the rate of fermentation of sugar by yeast-juice.

The following table gives results of three experiments. The estimations were made by digesting the yeast and liquid with nitric and sulphuric acids, and, after dispelling the nitric acid, reducing the arsenic acid with hydriodic acid. The liberated iodine was removed by titration with thiosulphate and the arsenious acid precipitated with sulphuretted hydrogen, collected on a tared filter, washed successively with water, alcohol and carbon bisulphide, dried at 100° and weighed. The results are expressed in terms of anhydrous sodium arsenate.

Table XII.—Diffusion of Sodium Arsenate.

| No. | Conditions. | | Yeast. | | Liquid. | | P. | $P_1$. | K. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial. | Final. | Initial. | Final. | | | |
| 164 | Concentration, 2·02 per cent. = 0·11 molar. Stood 20 hrs. in cold room | Total ... | 100·00 | 102·40 | 100·00 | 97·60 | | | |
| | | Arsenate | nil | 0·15 | 2·02 | 1·86 | 0·22 | 1·95 | 0·11 |
| 165 | ,, ,, | Total ... | 100·00 | 97·90 | 100·00 | 102·10 | | | |
| | | Arsenate | nil | 0·26 | 2·02 | 2·10 | 0·39 | 2·12 | 0·18 |
| 166 | Concentration, 3·35 per cent. = 0·18 molar | Total ... | 100·00 | 88·20 | 100·00 | 111·80 | | | |
| | | Arsenate | nil | 0·28 | 3·35 | 3·27 | 0·49 | 3·03 | 0·16 |

These results are essentially similar to those obtained with sodium chloride and ammonium sulphate, the factor K shows a fair degree of uniformity and indicates definite but very imperfect diffusion of the substance.

### *Summary and Conclusions.*

The early experiments on plasmolysis of yeast seemed to indicate that the envelope of the yeast-cell was impermeable by inorganic salts generally while it allowed of the ready diffusion of such substances as alcohol, acetone, and urea, which have been known to pass with ease through many forms of living protoplasm.

Quantitative estimations have shown the power of diffusion of alcohol to be very different from that of inorganic salts. On immersion of yeast in dilute alcohol, varying from 5 per cent. to 20 per cent., the ratio of the concentration within the cells to that of the liquid outside becomes practically constant, and independent of the absolute concentration. Alcohol is believed to diffuse quite readily into the cell, but at the same time this ratio is not unity, but a constant which deviates only slightly from 0·85. Probably the whole of the water in the cell, which is removed by drying at 98° C., is not available for diffusion of alcohol. The amount of water thus bound up, possibly as a constituent of the protoplasmic complex, appears to vary somewhat at different stages in the life-history of the cell, but the method was not considered sufficiently delicate to render further study of this interesting phenomenon advisable in this way.

All salts which have been tried have been taken up by yeast from moderately concentrated solutions, and in the cases of sodium chloride and ammonium sulphate even from dilute solutions. But, whereas with alcohol the amount entering the yeast during three hours was practically equal to the amount which entered on prolonged immersion, with these salts the process was a slow one. After three hours no sodium chloride had entered from a decimolar solution, and considerably less ammonium sulphate was found in the yeast than was the case after longer standing. From decimolar solution of sodium phosphate no entrance of phosphorus was appreciable even after 20 hours' standing, but from more concentrated solution, 0·3 molar, a well marked entrance was observed. Since phosphates are essential for the life of the yeast and are gradually assimilated and accumulated from very dilute solutions, the envelope must admit the necessary amount of these substances required by the cell for its metabolism. The amount thus absorbed during the time of these experiments would naturally be very small and indeterminable.

With regard to the entrance of salts, which the experiments have shown to occur, the following considerations are of interest. Since the yeast must of necessity be analysed as a whole, the question as to how far into the cells the various substances have penetrated must, at present, remain in doubt. While most salts do show some entrance into the cells, the factor which is taken as an expression of permeability is, except in the case of copper sulphate, comparatively small (0·1—0·25 as against 0·85 in the case of alcohol). It seems very probable that the apparent entrance of salts is a result of adsorption in the surface layers of the cell rather than absorption, or it may be that the salt particles are kept back by a differential septum according to the hypothesis of H. E. Armstrong (8), and that they remain in the interstices of such membrane.

The experiments with hexosephosphate are particularly interesting in this connection, since this substance is present in yeast and is readily hydrolysed and fermented by yeast-juice. The fact that when this substance is added to yeast there is no evidence whatever of its being fermented would seem to indicate that it had not been able to penetrate through to the seat of fermentative activity. It thus seems highly probable that the apparent entrance of this salt, which is well marked, is merely a surface phenomenon.

In conclusion, the author desires to express his best thanks to Dr. A. Harden, at whose instigation the work was commenced, and whose kindly interest and numerous suggestions have been highly esteemed.

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# *The Intrinsic Factors in the Act of Progression in the Mammal.*

By T. Graham Brown.

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(From the Physiological Laboratory of the University of Liverpool.)

CONTENTS.

PAGE
I. Introduction ........ 308
II. Operative Procedure ........ 312
III. Movements of Progression in the Low Spinal Preparation in which the Muscles are not De-afferented ........ 313
IV. Movements of Progression in the Low Spinal Preparation in which the Muscles are De-afferented ........ 314
V. Conclusions ........ 316
VI. Summary ........ 319

## I. *Introduction.*

Whilst the act of progression is being performed, the several limbs exhibit rhythmic movements of flexion and of extension. When any limb is in contact with the ground, it extends, and thus serves to propel the animal forwards. At the end of this act the limb is lifted from the ground by a movement of flexion, is carried forward, and finally is again placed upon the ground to repeat the cycle. During these phasic acts the dynamic balance of the neural centres is disturbed by two different kinds of peripheral stimuli.

In the first place, the discontinuous contact with the ground, and the synchronous distortion of the skin of the foot—determined by the weight of the animal then carried in part by that limb—produce changes in the activity of exteroceptive end-organs therein embedded, and discontinuous augmentations and diminutions of the stimuli originated in them.

In the second place, the backward and forward movements of the limb, and the activity of the muscles which execute them, produce changes in the state of the proprioceptive organs situated in the muscles, joints, and tendons which take part in the act.

The act of progression is one richly co-ordinated. Yet it has long been known that movements of the hind limbs, certainly those of progression, may be present in the "late spinal animal." A mechanism confined to the lumbar part of the spinal cord is therefore sufficient to determine in the hind limbs an act of progression, which is probably very nearly a normal one. As reflex movements of the hind limb, exactly similar to movements integrated in the act of progression, may be obtained by the artificial stimulation of exteroceptive or of proprioceptive end-organs, the suggestion has naturally arisen that the act of progression may be entirely determined by cyclic variations of the stimuli which arise in peripheral sense organs, and are themselves conditioned by the movements which they engender —in other words, that the act of progression is automatic and conditioned by the integration of reflex movements which follow each other successively, and each of which determines the stimulus which calls the following movement into being.

In discussing the nature of progression, Philippson* has laid stress upon the stimulation of the skin as a factor in the determination of the act. Before this, Sherrington† had observed that in the spinal dog and cat, the ipsilateral flexion reflex may be evoked, soon after the trans-section, by pressure so directed upwards upon the pads of the foot that the toe-joints are extended—the stimulus being comparable to the pressure of the ground in progression. But later, when the shock of the operation has passed, the same stimulus will evoke the ipsilateral extension reflex—the "extensor thrust"—if the stimulated limb be in a state of flexion. If it be extended, the reaction is one of flexion as before. Philippson believes that this reflex plays an important part in the mechanism and determination of progression. He supposes that the first contact of the limb with the ground evokes the extensor thrust. This reaction is reïnforced by the crossed extension which accompanies the flexion of the opposite limb. These two determine an increase of pressure of the foot upon the ground, and this peripheral exteroceptive stimulus then causes a reflex flexion of the same limb and an extension of the opposite limb, which then is about to come, in its turn, into contact with the ground. The former limb is now flexed and carried forward while the latter is in contact with the ground, and the stretching of the skin thus caused in the inguinal region determines the appearance of extension in the flexed limb. This extension brings the limb on to the ground, where the contact determines the extensor thrust. And so the cycle begins again. On this hypothesis, it is, however, difficult to explain the mechanism of Freusberg's‡ "mark-time" reflex. There the "late spinal" dog, when suspended free from the ground, performs movements of progression with its hind limbs. Philippson thinks that here the crossed extension due to the ipsilateral flexion, combined with the inguinal stretching of the skin and with the action of association paths in the lumbar cord, are sufficient to produce the phenomenon in the absence of contact with the ground.

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* 'L'autonomie et la centralisation dans le système nerveux des animaux,' Bruxelles, 1905.

† 'Roy. Soc. Proc.,' 1900, vol. 66, p. 66.

‡ 'Arch. f. d. ges. Physiol.,' 1874, vol. 9, p. 358.

The difficulty in explaining this phenomenon has been emphasised by Sherrington.* He points out that, in the intact animal (cat, dog), severance of all the sensory nerve trunks directly distributed to all four feet up to and above the wrists and ankles scarcely impairs the act of progression. He also notes that reflex stepping is not annulled, or even obviously impaired, by severance of all the various cutaneous nerves of the limb. And stretching of the prominent fold of skin which runs along the outer edge of the groin cannot be of essential importance in the act, because cocainisation of this region does not interfere with reflex stepping. The extensor thrust may also be abolished—by that division of the sensory nerves of the foot described above—without noticeably changing the acts of the walk and trot. He therefore concludes that the intrinsic stimuli for reflex stepping of the limb are not referable to any part of the skin of the limb.

In continuation of his work on proprioceptive reflexes,† Sherrington finds in the sensory end-organs of the muscles themselves the seat of the intrinsic stimuli for reflex stepping.‡ He considers that the mode of process in reflex walking is as follows: The spinal step is a rhythmic reflex which may be excited by continuous stimuli applied either to the cross-section of the divided spinal cord or to various peripheral points outside the limb itself. The generating stimulus is continuous, but the movement of the limb is determined by the alternate action of two antagonistic reflexes. The primary stimulus sets one of these in action. This act generates in that limb a proprioceptive reflex antagonistic to itself. The proprioceptive reflex interrupts the primary reflex, and in this interruption abolishes the stimulus which engendered itself. The primary reflex is then reconstituted and again calls forth the interrupting reflex, and so on. The secondary reflex is determined by the combination of three main factors—centripetal impulses from the deep structures moved passively by the primary reflex (joints, etc.); centripetal impulses from the muscles which move actively in the primary reflex; and the central change underlying "rebound." The phenomenon of "reflex reversal" and the "extensor thrust" may also play a part.

Of particular significance is this factor of "central rebound." Sherrington§

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* 'Journ. Physiol.,' 1910, vol. 40, p. 28.

† 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 552; 'Q. Journ. Exp. Physiol.,' 1909, vol. 2, p. 109.

‡ 'Journ. Physiol.,' 1910, vol. 40, p. 28.

§ 'Roy. Soc. Proc.,' B, 1905, vol. 76, p. 160; 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 478.

has noted that the flexion reflex, for instance, is often terminated after cessation of the exciting stimulus by an active phase of extension; and that the individual muscles which are in a state of inhibition during the application of the stimulus contract suddenly after its termination. That this phenomenon is not due to a proprioceptive stimulus generated in the muscles which take part in that primary flexion reflex—or at any rate is not solely conditioned by such a stimulus—is shown by Sherrington's observation* that the rebound contraction—"successive spinal induction"—may be obtained in the de-afferented preparation.

Another point of interest and importance is the partial similarity of the act of progression with that of the scratch. Sherrington† has noted this similarity, and the present author‡ has shewn that in the anæsthetised rabbit the one state may immediately follow upon the other, and that there is indication in some cases that the two may blend for a time. This partial similarity is suggestive. Sherrington§ found that in the scratch-reflex the flexion of the thigh did not completely relapse during each brief extension of the phasic act—that there was always a certain amount of maintained flexion. In certain states, as the present author|| was able to shew for the guinea-pig, the two factors in the scratch may be separated. And of these two factors one is a state of maintained flexion while the other is a discontinuous inhibition of that state. In the scratching phenomenon described by the present author¶ as occurring in the guinea-pig under anæsthesia there is an alternation of the state of scratching from one hind limb to the other. At any one time the state of maintained flexion complicated by rhythmic inhibition is accompanied in the crossed hind limb by a state of maintained inhibition of flexion. He has suggested that the rhythmic inhibition during maintained flexion and the maintained inhibition of flexion which immediately succeeds in the same hind limb may be expressions of one and the same activity; and that they may, in effect, be conditioned by variations in the mutual influence of interacting spinal centres. The suggestion in reality is that the locus of the inhibitory factor is central, and that it is not of essential peripheral origin from proprioceptive stimuli. For the scratch this is in accordance with a previous observation of Sherrington** that the

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* 'Q. Journ. Exp. Physiol.,' 1909, vol. 2, p. 109.

† 'Journ. Physiol.,' 1910, vol. 40, p. 28.

‡ 'Q. Journ. Exp. Physiol.,' 1911, vol. 4, p. 151.

§ 'Journ. Physiol.,' 1906, vol. 34, p. 1.

|| 'Q. Journ. Exp. Physiol.,' 1910, vol. 3, p. 21; 'Q. Journ. Exp. Physiol.,' 1911, vol. 4, p. 19.

¶ 'Q. Journ. Exp. Physiol.,' 1910, vol. 3, p. 21.

** 'Journ. Physiol.,' 1906, vol. 34, p. 1.

de-afferented hind limb of the cat may be made to scratch. Although this is perhaps not conclusive—in view of the possible influence of stimuli which arise in the opposite hind limb—he and the present author have recently demonstrated at a meeting of the Physiological Society a cat in which scratching may be elicited although both hind limbs have been completely de-afferented.

The phasic alternation of maintained flexion in the scratching phenomenon of the guinea-pig under anæsthesia in many ways resembles the phasic alternation of flexion in the act of progression, and has suggested for some time to the present author that the act of progression may, too, be essentially a central and not a peripheral phenomenon.

## II. *Operative Procedure.*

In these experiments the animal used was the decerebrate cat, and the movements of the isolated *tibialis anticus* and *gastrocnemius* were recorded. Before decerebration a loose ligature was placed round the spinal cord at the level of the 11th, 12th, or 13th pair of thoracic spinal roots; and all the posterior spinal roots caudal to, and including, the 6th lumbar root were divided upon the side of the recording muscles. In the limb of that side all the other muscles were put out of action by the severance of their motor nerves or of their substance; there remained in action only the muscles supplied by the branches of the popliteal nerves in the leg, and these, besides having their tendons cut, were de-afferented by the section of the lumbar roots. The long saphenus nerve, a purely afferent one, was left intact for another purpose. In the opposite hind limb all the muscles were de-afferented by the section of their nerves or by the section of their substance; there remained of the nerves of that limb only the long saphenus nerve.

In effect this procedure completely de-afferented the two hind limbs, including the muscles whose movements were to be recorded.

To record the muscular movements the leg was firmly fixed in steel clamps, and the recording muscles wrote upon the myograph by means of light aluminium levers to which their tendons were attached by threads.

An interval of about five hours was allowed to elapse between the end of the operative procedure described above and the experiment about to be described.

It is hardly necessary to state that up to the time of decerebration the animal was kept completely under the influence of the anæsthetic.

## III. *Movements of Progression in the Low Spinal Preparation in which the Muscles are not De-afferented.*

Before passing to the consideration of the movements of progression in de-afferented muscles it is well to examine briefly the movements which occur in the same preparation when the muscles have not been de-afferented. The procedure of operation was that described above, with the exception that the lumbar posterior spinal roots were not divided.

When such a decerebrate preparation is rendered a low spinal preparation by the rapid severance of the spinal cord at or about the level of the 12th thoracic segment the recording muscles shew movements undoubtedly those of progression.

Three periods may be distinguished in typical instances of the reaction thus evoked as movement at the ankle.

Immediately after the section of the cord there is a period during which the flexor muscle, *tibialis anticus,* remains more or less in a state of maintained contraction. The maintenance of this contraction is not perfect. There are usually phases of incomplete relaxation. These become more frequent and more complete towards the end of this period, and at its termination the relaxation phases are complete but rather irregular in rhythm. During the first period the extensor, *gastrocnemius,* plays little part, and may exhibit no movement at all. Towards the end *gastrocnemius* begins to exhibit movements synchronous with the relaxations of *tibialis anticus.*

In the second period the movements of the antagonistic muscles are very regular and alternate. The flexor record demonstrates regular phases of contraction separated by regular phases in which the muscle remains relaxed. The extensor record exhibits contractions of the muscle synchronous with the relaxation phases of *tibialis anticus.* These contractions are of rapid initiation and short duration. They seem to commence at the moment in which the movement of the flexor changes from contraction to relaxation, and they resemble the "extensor rebound" observed after the cessation of a state of extensor inhibition.

In the third period of the reaction the movements of the flexor become smaller in extent and appear at greater intervals of time. There is a change in the type of movement of the extensor. The extensor record changes completely in appearance. The muscle tends to remain in a state of contraction, and this is broken by phases of relaxation which are synchronous with, but commence before, the contractions of the flexor.

This third period of the reaction is the terminal one. The movements of the flexor cease. The extensor remains in a state of contraction, still, however, complicated by phases of relaxation. These finally cease, and the reaction terminates with the extensor in a condition of maintained and unbroken contraction.

### IV. *Movements of Progression in the Low Spinal Preparation in which the Muscles are De-afferented.*

When the spinal cord is severed in the same manner in the de-afferented preparation movements of progression may be obtained, and these are similar to the movements observed in the preparation in which the afferent nerves of the recording muscles are intact.

In the record illustrated (see figure) the cord was cut approximately at the point marked X in the figure on the signal line below (X, X also on the two tracings). The first period lasts approximately up to the ordinates marked 1. The upper tracing shews a state of maintained flexion broken by more or less incomplete relaxations of short duration. The contraction of *gastrocnemius* shewn in the lower tracing is unusual. The second period lasts approximately up to the ordinates marked 8. The acts of flexion and extension are very regular. Examination of the ordinates 4, 5, 6, and 7 shews that the commencement of flexion is accompanied by *gastrocnemius* relaxation; the change from contraction to relaxation at the top of the *tibialis anticus* "beat" is accompanied by a contraction of *gastrocnemius*; this does not last up to the point at which flexion recommences. This contraction of *gastrocnemius* strongly resembles the contraction of "rebound." The third period commences at or about the point marked by ordinates 8, and persists up to the end of the record. After the contraction of *gastrocnemius* marked by ordinates 9, the curve begins to rise slowly. This is broken by a relaxation which commences before the *tibialis* contraction marked by ordinates 10. This flexion movement is small, and is not accompanied at its change to relaxation by a contraction of *gastrocnemius.* Thenceforth the *gastrocnemius* tracing exhibits only periods of relaxation synchronous with the *tibialis* contractions, and no rebound contraction. After ordinates 12 the *gastrocnemius* remains in maintained contraction.

The records thus shew the same three periods observed in afferent-present reactions.

In the first period the maintained contraction of the flexor muscle—complicated by more or less incomplete phases of relaxation—is present. This at first has sometimes been accompanied by little or no movement of the extensor; in other cases this latter movement has been present.

The second period seems to be characterised by a greater regularity than is usual in preparations in which the afferent arcs are unbroken. The movement

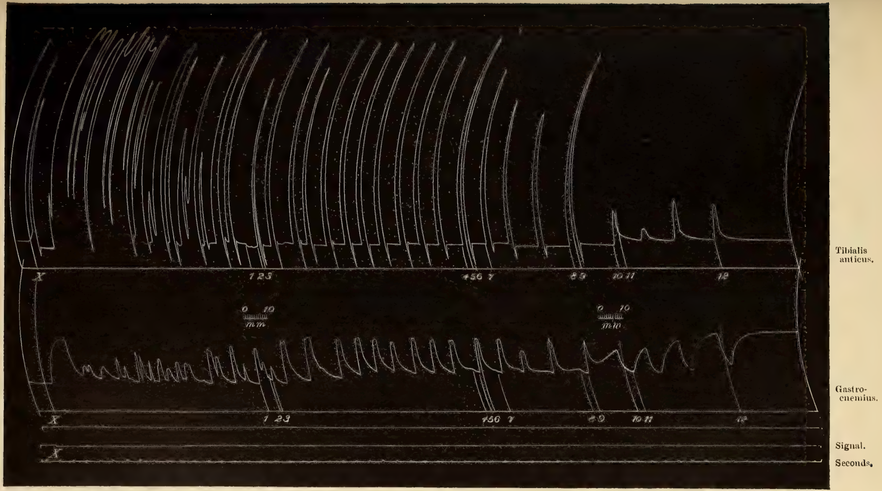

FIG. 1.—Decerebrate cat, June 19, 1911. Recording muscles de-afferented, and all others de-afferented and paralysed. Record of movements of progression as evidenced in *tibialis anticus* (upper tracing) and *gastrocnemius* (lower tracing) upon cutting the cord completely across between the levels of origin of the twelfth and thirteenth pair of thoracic spinal roots. The cord was cut six and a half hours after the decerebration. In the tracings the rise of the curve denotes contraction, the fall relaxation; the record is to be read from left to right; seconds are marked below; ordinates, marked 1, 2, 3, etc., mark corresponding points in the two tracings; and millimetre scales have been reduced in proportion with the record.

of the extensor is strongly reminiscent of rebound contraction. It does not persist for the whole of the duration of the synchronous flexor relaxation, but relaxes soon. There is then sometimes an additional relaxation synchronous with the contraction of the flexor. The termination of this flexor contraction is then succeeded by the extensor contraction—and so on.

In the third period of the reaction the rebound-like contraction of the extensor disappears and is replaced by a condition of maintained contraction which is broken by relaxations synchronous with the flexor contractions. These are much smaller than before and gradually disappear. The transition of the type of flexor contractions is sometimes sudden. The period finally ends with a maintained and unbroken contraction of the extensor.

It may be said that there is no great difference between the movements of progression as evidenced in the de-afferented muscles and those not de-afferented. The greater regularity of the movements as observed in the former preparation may possibly be due to the absence of the peripheral part of the mechanism. But it is also possible that the difference is an accidental one; for records as regular have been obtained from the preparation in which the muscles are not de-afferented, although the average regularity is less.

## V. *Conclusions.*

These experiments show that the *phasing of the acts of progression is determined neither by the peripheral skin stimuli nor by the self-generated proprioceptive stimuli of the muscles which take part in them.*

The section of the spinal cord generates an arrhythmic stimulus. This causes the contraction of certain limb muscles. In the preparation used, of these the recording muscles are the only mobile parts of the two limbs. The characteristic alternating contraction of the two antagonists cannot be determined by their own contraction and the consequent setting up of a series of refractory phases in the activity of the centres by means of the stimulation of a sensory apparatus contained in the muscles, because the afferent nerves which arise in these muscles were put out of action in the preparation used. Not only must the locus of the changes which condition the refractory phase of progression be in the spinal cord, but *the mechanism which determines them must also be central.*

There are, therefore, two points of interest in connection with the mechanism of progression—the question of the nature of the central changes in activity, and the question of the part played in the act by the proprioceptive mechanism. The stimuli which arise in the skin probably play but a small part in the act, and then are of importance only in certain of its types and not in all.

The evidence given by the records throws some light upon the problem of the nature of the central activities. In a typical example there is first a period in which a state of maintained flexion, although a broken one, is the predominant feature. The terminating period of the reaction is one characterised by maintained extension—at the end unbroken. Between these there lies a period in which the phasing of the act is most perfect. This period—lying as it does intermediate between those of maintained flexion and of maintained extension—may be regarded as a period of balance.

Any hypothesis regarding the nature of the central activities must at present be tentative, but this appearance of "balance" supports a view put forward by the author in previous papers.* As regards the act of progression the central mechanism may be regarded as consisting of antagonistic centres. In one hind limb it may be supposed that the one of these determines a state of maintained flexion and a concomitant state of maintained inhibition of extension, while the other determines a state of maintained extension and a concomitant state of inhibition of flexion. It is inessential at present whether the lumbar centres are two in number and situate in opposite sides of the spinal cord; or whether they are four in number and situated in antagonistic pairs on each side of the cord; or whether they are more than four in number.† All that it is desired to insist upon here and now is that the centres are paired, and that each pair consists of antagonistic opposites.

If this be granted, and if there be presumed some such state as "fatigue"—in its broadest sense—accompanying the expression of activity of either of the antagonistic centres, it is possible to frame a tentative hypothesis of the nature of the central changes which condition the phasic act of progression.

It may then be supposed that some neural disturbance rearranges the conditions of activity of the antagonistic centres in such a manner as to destroy their balance. The one centre then expresses its activity in a movement—for example, flexion—of the limb, and at the same time inhibits the activity of its antagonistic centre. But this expression of activity is accompanied by a state of "fatigue," which progresses in extent and tends to restore the balance of the centres. At the same time the inhibition of the other centre by the first decreases in efficiency. The point then arrives when the second centre is no longer inhibited efficiently. But there is then following upon the inhibitory depression an exaltation of its activity—"rebound." The balance is therefore not only regained but overshot. The exhibition of activity by the second centre then determines the contraction of the antagonistic group of muscles, and at the same time there is an inhibition of the activity of the first centre. The "fatigue" which accompanies this activity of the second centre then sets in. The balance is restored, but again overshot. And so the act proceeds.

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* 'Q. Journ. Exp. Physiol.,' 1910, vol. 3, p. 21; 'Q. Journ. Exp. Physiol.,' 1911, vol. 4, p. 19.

† For instance, there may be antagonistic factors (contraction and relaxation producing) in one and the same centre. Thus the activity of such a centre as that for *tibialis anticus* might be exhibited either as contraction or as relaxation of the muscle.

The phenomenon of rebound, which Sherrington* has shewn to be of central locus, may play a very important part in the swinging of balance between the spinal centres. And the phenomena which underlie the phasic act of progression may be likened to the beating of a pendulum. The activity exhibited may remain for a time flexion, may then swing back to the neutral point of spinal balance, but may overshoot this and become extension activity, may then swing back past the neutral point into flexion activity—and so on.

There remains the question of the part played by the proprioceptive mechanism in the act.

There can be no question of its importance nor of its suitability to augment the central mechanism. It cannot, however, be regarded as determining the refractory phases in the act. Its part must be regulative, not causative.

A purely central mechanism of progression ungraded by proprioceptive stimuli would clearly be inefficient in determining the passage of an animal through an uneven environment. Across a plain of perfect evenness the central mechanism of itself might drive an animal with precision. Or it might be efficient, for instance, in the case of an elephant charging over ground of moderate unevenness. But it alone would make impossible the fine stalking of a cat over rough ground. In such a case each step may be somewhat different to all others, and each must be graded to its conditions if the whole progression of the animal is to be efficient. The hind limb, which at one time is somewhat more extended in its posture as it is in contact with the ground, in another step may be more flexed. But the forward thrust it gives as its contribution to the passage of the animal must be of a comparatively uniform degree in each consecutive step. It may only be so if it is graded by the posture of the limb when in contact with the ground, and by the duration of its contact with the ground. This grading can only be brought about by peripheral stimuli. Of these we must regard the proprioceptive stimuli from the muscles themselves as the most important, and *the part which they play is essentially the regulative—not the causative.*

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* 'Q. Journ. Exp. Physiol.,' 1909, vol. 3, p. 109.

This work has been done during the tenure of a Carnegie Fellowship, and the expenses of the research have been defrayed by a grant from the Carnegie Trust.

## *Summary.*

1. By means of a stimulus (namely, section of the spinal cord) central in application, although remote from the local centre, the act of progression may be induced in muscles de-afferented by the cutting of their appropriate posterior spinal roots. It occurs thus after all the muscles of both hind limbs have been de-afferented, and all but the recording pair have been put out of action by motor paralysis.

2. The act of progression as exhibited by these muscles and thus induced scarcely differs, if indeed it differs at all, from the act similarly induced when the afferent arcs of the recording muscles have not been broken.

3. In either case the reaction, as evidenced in movement at the ankle-joint, shews three periods. In the first the record is characterised by a state chiefly of maintained flexion. In the last there is a state characterised by maintained extension. Intermediate between these there is a period of "balance," in which the movements of progression are most perfect.

4. *The rhythmic sequence of the act of progression is consequently determined by phasic changes innate in the local centres, and these phases are not essentially caused by peripheral stimuli.*

5. *The proprioceptive stimuli which are generated by the contraction of muscles taking part in the act (when the appropriate posterior spinal roots are intact) play a regulating and not an intrinsic part in the act. Their chief importance may be in the grading of the individual component movements to the temporary exigencies of the environment.*

# *An Inquiry into the Influence of the Constituents of a Bacterial Emulsion on the Opsonic Index.*

By A. F. Hayden, M.B., B.S., F.R.C.S., I.M.S., and W. Parry Morgan, M.A., B.Sc., M.B.

(Communicated by Sir A. E. Wright, F.R.S. Received August 24,—
Read November 2, 1911.)

(From the Inoculation Department, St. Mary's Hospital.)

In preparing an emulsion of bacteria for opsonic estimations it is necessary to break up the masses so far as possible into their constituent bacterial elements and then to separate these from any clumps by centrifugalisation. The rate at which the suspended particles of an emulsion settle depends not only on the centrifugal force applied, but also on the fineness of the particles, and therefore on the efficiency of the method of breaking up the masses. If this is not efficient the suspended matter will fall in the form of coarse particles, leaving a relatively clear supernatant fluid containing very little in suspension.

In the case of a tubercle emulsion we find that the best results are given by triturating a small quantity of dried bacilli with a pestle and mortar of which the grinding surfaces have the same curvature; using these, five minutes' grinding is ample. The mass of dried bacilli is first ground up in the dry state and then made into a paste with a little 1-per-cent. saline (the strength used in all our experiments). The crude emulsion is then made by taking up the paste with 1 to 1½ c.c. of saline. When this emulsion is thoroughly centrifugalised it separates out into a deposit and an opaque supernatant fluid which is practically free from bacilli but which contains a considerable amount of bacterial detritus. If this supernatant fluid be pipetted off and the deposit again mixed with fresh saline and thoroughly centrifugalised, the second supernatant fluid will contain much less detritus and will be correspondingly clearer. By repeating this process several times it is possible to get a supernatant fluid which is almost clear and free from detritus. The deposit will then consist wholly of washed bacteria.

It is thus seen that the usual bacterial emulsions which are employed for measuring opsonic power consist of three elements, the saline used as a menstruum, bacillary detritus, and intact bacilli.

We have set ourselves the problem of determining the effect of the bacillary detritus on phagocytosis and its influence in the estimation of the opsonic index. With this end in view we have sought answers to the following questions.

### *What is the Effect of Removing Detritus from a Bacterial Emulsion and of Adding it to such an Emulsion?*

*Experiment* 1.—An emulsion giving with the usual technique a count of 0·5 per cell in 15 minutes was centrifugalised; the supernatant fluid containing much detritus was pipetted off and an equal volume of saline added and shaken up with the deposit. The resulting emulsion then gave a count of 1·9 per cell. *Thus by removing some of the detritus the phagocytosis was increased about four times.*

*Experiment* 2.—Two phagocytic mixtures were made, consisting of equal volumes of washed corpuscles; suspension of washed bacilli; serum; and, in the one case, saline, and in the other, suspension of bacillary detritus.

The following were the counts:—

| (1)<br>With saline. | (2)<br>With suspension of detritus. |
|---|---|
| 1·61 | 0·62 |

Similar experiments gave the following counts:—

| | (1) | (2) |
|---|---|---|
| (*a*) ............ | 4·92 | 3·17 |
| (*b*) ............ | 2·05 | 1·40 |
| (*c*) ............ | 1·65 | 0·64 |

*Thus*—and this is the converse of the conclusion above arrived at—the *addition of detritus causes a well marked reduction in the phagocytosis.*

### *Is this Reduction Due to an Effect Exerted by the Bacillary Detritus on the Phagocytes, or on the Serum?*

*Experiment* 2B.—Three phagocytic mixtures were taken—equal volumes of corpuscles, suspension of washed bacilli, and—

(*a*) In the first mixture saline and serum.

(*b*) In the second, suspension of detritus and serum.

(*c*) In the third, two volumes of mixture of equal volumes of the suspension of detritus and serum, previously incubated for 20 minutes.

The following were the phagocytic counts:—

| (*a*) | (*b*) | (*c*) |
|---|---|---|
| 2·05 | 1·40 | 0·37 |

And a similar experiment gave

| | | |
|---|---|---|
| 1·65 | 0·64 | 0·27 |

*Experiment* 3.—The following phagocytic mixtures were used:—

(*a*) Equal volumes of
- Corpuscles.
- Suspension of bacilli.
- Two volumes of a mixture of equal parts of a suspension of detritus and serum which had been previously incubated for 20 minutes.

(*b*) Equal volumes of
- Corpuscles.
- Suspension of detritus.
- Two volumes of an incubated mixture of the suspension of bacilli and serum.

(*c*) Equal volumes of
- Corpuscles.
- Suspension of detritus, and
- Two volumes of an incubated mixture of saline and serum.

The following were the phagocytic counts:—

| | (*a*) | (*b*) | (*c*) |
|---|---|---|---|
| Patient i......... | 0·15 | 1·59 | 1·20 |
| Normal i......... | 0·22 | 1·81 | 1·09 |
| Patient ii......... | 0·19 | 1·37 | |
| Normal ii......... | 0·19 | 1·74 | 1·20 |
| Patient iii......... | 0·20 | 1·36 | 1·12 |

These experiments supplement the previous ones, in the respect that they show that the addition of detritus diminished phagocytosis by removing the opsonic power from the serum—in other words, that the detritus functions as a receptor. And we may conveniently speak of the receptors of the detritus as free receptors, in contrast to the fixed receptors of the intact bacilli.

Column (*a*) shows that the serum, when digested first with the free receptors, will combine with these to the practical exclusion of the fixed receptors which are afterwards added. Column (*b*) shows that the serum, when digested first with the fixed receptors, will now combine by preference with these, and Column (*c*) that, when free and fixed receptors are present, the serum at one and the same time will tend to combine more impartially with both.

### *How does the Absence or Presence of Detritus in the Bacterial Suspension Influence the Opsonic Index which is arrived at?*

*Experiment* 4.—Indices were estimated by using phagocytic mixtures of—

Corpuscles,
Suspension of bacilli, and
Serum.

And in (i) Saline, and
(ii) Suspension of detritus.

The following were the results:—

| | (1) Saline. | | (2) Suspension of detritus. | |
|---|---|---|---|---|
| | Phagocytic count. | Index. | Phagocytic count. | Index. |
| Normal serum | 1·61 | 1·00 | 0·62 | 1·00 |
| Patient's serum | 1·50 | 0·93 | 0·20 | 0·31 |
| A similar experiment gave | | | | |
| Normal serum | 2·47 | 1·00 | 2·14 | 1·00 |
| Patient's serum | 1·99 | 0·81 | 0·63 | 0·29 |

The following method of experimentation was also resorted to:—

*Experiment* 5.—Indices were estimated with two emulsions—(A) consisting of a suspension of washed bacilli, (B) of a suspension containing detritus.

The following were the results:—

| | (A) | | (B) | |
|---|---|---|---|---|
| | Phagocytic count. | Index. | Phagocytic count. | Index. |
| Normal 1<br>,, 2 | $\left.\begin{array}{l}1{\cdot}30\\1{\cdot}18\end{array}\right\}1{\cdot}24$ | 1·00 | $\left.\begin{array}{l}1{\cdot}53\\1{\cdot}70\end{array}\right\}1{\cdot}61$ | 1·00 |
| Patient 1 | 1·40 | 1·12 | 1·44 | 0·84 |
| ,, 2 | 1·08 | 0·86 | 1·12 | 0·69 |
| ,, 3 | 1·10 | 0·88 | 0·75 | 0·46 |
| ,, 4 | 1·15 | 0·92 | 0·82 | 0·51 |
| ,, 5 | 1·35 | 1·08 | 1·98 | 1·23 |

A similar experiment with the serum of another patient and two emulsions, (*a*) containing some detritus, and (*b*) containing a larger quantity of detritus, gave the following results:—

| | (A) | | (B) | |
|---|---|---|---|---|
| | Phagocytic count. | Index. | Phagocytic count. | Index. |
| Patient | 2·77 | 1·18 | 3·24 | 1·63 |
| Normal i.<br>,, ii. | $\left.\begin{array}{l}2{\cdot}40\\2{\cdot}28\end{array}\right\}2{\cdot}24$ | 1·00 | $\left.\begin{array}{l}1{\cdot}90\\1{\cdot}84\end{array}\right\}1{\cdot}87$ | 1·00 |

In this last experiment, the appearance as regards the degree of opacity, and so the quantity of detritus, in the emulsion A, was such as to lead us to believe that, had a serum with a low index been tested with it, a low index would have been obtained. The emulsion B was far more opaque than A, and so a further experiment was performed. Three emulsions were prepared, using such quantities of dried tubercle bacillus as to obtain varying amounts of detritus: (A) containing a small quantity; (B) a larger quantity; (C) a very large quantity.

Indices were estimated with these three emulsions, using two normal sera and two patients' sera, one whose index was expected to be high, and another whose index was usually low. The following were the results:—

| | (A) | | (B) | | (C) | |
|---|---|---|---|---|---|---|
| | Phagocytic count. | Index. | Phagocytic count. | Index. | Phagocytic count. | Index. |
| Normal i …… | 1·44 } 1·37 | 1·00 | 2·00 } 1·83 | 1·00 | 1·84 } 1·71 | 1·00 |
| „ ii …… | 1·30 | | 1·66 | | 1·58 | |
| Patient i …… | 1·66 | 1·21 | 2·19 | 1·19 | 3·44 | 2·01 |
| „ ii …… | 0·92 | 0·67 | 1·12 | 0·61 | 1·00 | 0·58 |

And a repetition of this experiment, using two different emulsions—(A) containing a moderate quantity of detritus, and (B) containing a large quantity—and using the sera of three other patients, gave the following results:—

| | (A) | | (B) | |
|---|---|---|---|---|
| | Phagocytic count. | Index. | Phagocytic count. | Index. |
| Normal i…………………… | 1·38 } 1·41 | 1·00 | 1·28 } 1·37 | 1·00 |
| „ ii…………………… | 1·44 | | 1·47 | |
| Patient i…………………… | 1·55 | 1·10 | 1·93 | 1·41 |
| „ ii…………………… | 1·73 | 1·22 | 2·53 | 1·85 |
| „ iii…………………… | 0·48 | 0·33 | 0·48 | 0·34 |

Thus, when little or no detritus was contained in the emulsion, the indices were in all cases within normal limits. When detritus was present in moderate quantities, differences emerged between bloods of normal and subnormal opsonic power, but supernormal bloods were not differentiated from normal bloods. When emulsions which contained larger quantities of detritus were employed, both subnormal and supernormal bloods were clearly differentiated from normal bloods.

### *Why does a Suspension of Washed Bacilli such as used in the foregoing Experiments fail to give Satisfactory Indications in Opsonic Power? And why does the Addition of Detritus improve it in this respect?*

Reflection shows that, when we are testing a series of sera with a view to eliciting differences in their bactericidal agglutinating or opsonic powers, we cannot expect that the full differences will be revealed unless the bacterial suspension which we employ contains more microbes than the strongest blood is competent to kill, agglutinate, or opsonise. And we can be certain that if the bacterial suspension contains only such number of microbes as the weakest blood is able to kill, agglutinate, or opsonise, no differences can be expected to emerge between the several bloods. We cannot, for instance, in the case when we are dealing with a batch of sera, of which the strongest is competent to kill 6,000,000 typhoid bacilli, while the weakest is able to kill only 600,000 bacilli, expect to elicit any differences of power with a suspension which contains no more than 600,000 bacilli. Nor could we, if we employed a suspension containing only 3,000,000, hope to differentiate between bloods that can kill 3,000,000 and bloods which can kill up to 6,000,000.

In the same way, when we are dealing with a batch of sera, of which the strongest would be competent to opsonise sufficient microbes to give an average ingest of 10 bacilli per cell, and the weakest only enough to give an average ingest of 2·5 microbes per cell, we cannot expect to bring out any differences between the bloods with a suspension which could provide at most 2·5 microbes per cell. Nor again, if we employ an emulsion which could not provide more than five bacilli per cell, could we hope to differentiate between bloods which could be competent to opsonise sufficient microbes to give a count of 5 per cell, and bloods which would be able to opsonise sufficient microbes to give us a count of 10 bacilli per cell.

It might, in view of this reasoning, seem as if the only satisfactory suspension would in the bactericidal test above mentioned be a suspension containing six or more millions of typhoid bacilli, and for the opsonic test a suspension which would give an average ingest of 10 microbes per cell. But this is not so.

All that is required is that each suspension should contain a quantity of receptors equal to that which will be contained in these suspensions of the required strengths, and Wright and Windsor* have shown in connection with the bactericidal power that if the bloods are partially depleted of their bactericidal power by the addition of receptors in the form of dead typhoid bacilli, quite weak suspensions of living typhoid bacilli can be used for the purposes of a differential test.

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* 'Journal of Hygiene,' Oct., 1902, vol. 2, No. 4, and 'Studies of Immunisation,' p. 45.

The same thing holds, as our experiments have shown, in connection with the opsonic power. If, as in Experiments 4 and 5, we partially deplete our sera of opsonic power by the addition of detritus to the phagocytic mixture, we can use quite weak suspensions of intact bacilli for the purposes of the differential opsonic test, and this will give us a sensitive indicator which will keep the bacterial ingest within the limits which will allow of its being accurately enumerated.

And we have here also a new and important point. If only a small quantum of detritus is present it will be possible to differentiate subnormal from normal indices, but not supernormal from normal; and not until we have sufficient detritus can we hope to differentiate both subnormal and supernormal from normal. We can, in short, only obtain a perfect indicator of differences in opsonic power by preparing our emulsions in such a way as to contain this sufficiency. This is clearly brought out in the latter experiments of Series V. And further, perhaps the most important result of these experiments is to explain the fact that with one and the same blood very different opsonic indices may be obtained by different workers, and even by the same worker at different times.

# *The Morphology of* Trypanosoma gambiense (Dutton).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.

(Received September 29,—Read November 2, 1911.)

[PLATE 13.]

## INTRODUCTION.

This species, like *Trypanosoma brucei*, is markedly dimorphic. In size and general appearance also these two species so closely resemble one another that one might easily believe them to be varieties of the same species. There are, however, some slight differences in morphology, which will be described below; but whether these differences will bear the test of more extended observations remains to be seen. It may be noted that the trypanosomes described come from Uganda, and are not mixed up with strains from the Congo or Rhodesia.

### A. *Living, Unstained.*

*Trypanosoma gambiense* also resembles *Trypanosoma brucei* in having little or no translatory power when viewed alive in the field of the microscope.

### B. *Fixed and Stained.*

The blood films were, as a rule, fixed, stained and measured as previously described in the 'Proceedings.'*

*Length.*—The following table gives the length of this trypanosome as found in man, chimpanzees, monkeys, oxen, antelope and rats, 1,000 trypanosomes in all. (See Table I.)

From the following table it would appear that *Trypanosoma gambiense* is somewhat smaller than *Trypanosoma brucei*, which was found to average 23·2 microns in 1,000 individuals, with a maximum length of 38 and a minimum of 13. (See Table II.)

Great differences are sometimes found in the average length of the trypanosomes in the same individual. For example, in Experiment 114, man (J. M.), on one day, at the beginning of his illness, the average of 20 trypanosomes was only 17·0 microns; whereas, on another day, at a later date, this rose to 25·8 microns.

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* 'Roy. Soc. Proc.,' B, 1909, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of *Trypanosoma gambiense*, Uganda Strain.

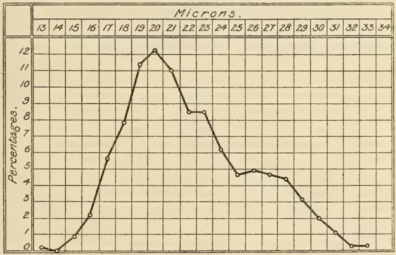
| No. of expt. | Animal. | Method of fixing. | Method of staining. | In microns. | | |
|---|---|---|---|---|---|---|
| | | | | Average length. | Maximum length. | Minimum length. |
| 114 | Man (J. M.) | Osmic acid | Leishman | 17·0 | 20·0 | 15·0 |
| 114 | ,, ,, | ,, | ,, | 25·8 | 30·0 | 20·0 |
| 103 | ,, (L. S.) | ,, | ,, | 25·0 | 32·0 | 16·0 |
| 103 | ,, ,, | ,, | ,, | 25·7 | 32·0 | 19·0 |
| — | ,, | ,, | ,, | 28·3 | 33·0 | 21·0 |
| — | Chimpanzee | ,, | ,, | 22·0 | 29·0 | 18·0 |
| — | ,, | ,, | ,, | 24·6 | 33·0 | 19·0 |
| — | ,, | ,, | ,, | 23·7 | 33·0 | 18·0 |
| 579 | Monkey | ,, | Giemsa | 22·5 | 31·0 | 17·0 |
| 674 | ,, | ,, | ,, | 21·5 | 28·0 | 15·0 |
| 976 | ,, | ,, | ,, | 24·8 | 29·0 | 18·0 |
| 976 | ,, | ,, | ,, | 22·8 | 31·0 | 17·0 |
| 1417 | ,, | ,, | ,, | 21·0 | 25·0 | 18·0 |
| 1418 | ,, | ,, | ,, | 21·4 | 26·0 | 16·0 |
| 1423 | ,, | ,, | ,, | 19·6 | 26·0 | 17·0 |
| 1423 | ,, | ,, | ,, | 20·3 | 30·0 | 16·0 |
| 1424 | ,, | ,, | ,, | 23·7 | 30·0 | 18·0 |
| 1424 | ,, | ,, | ,, | 22·7 | 29·0 | 17·0 |
| 1685 | ,, | ,, | ,, | 22·2 | 28·0 | 19·0 |
| 1685 | ,, | ,, | ,, | 23·5 | 30·0 | 17·0 |
| — | ,, | ,, | ,, | 20·2 | 27·0 | 17·0 |
| 1277 | ,, | ,, | ,, | 24·7 | 31·0 | 18·0 |
| 2483 | ,, | ,, | ,, | 26·2 | 30·0 | 20·0 |
| 982 | Ox | ,, | ,, | 19·3 | 23·0 | 17·0 |
| 1462 | ,, | ,, | ,, | 20·1 | 29·0 | 17·0 |
| 1462 | ,, | ,, | ,, | 18·9 | 23·0 | 16·5 |
| 1633 | ,, | ,, | ,, | 19·0 | 26·0 | 16·0 |
| 1633 | ,, | ,, | ,, | 19·0 | 25·0 | 15·0 |
| 1201 | ,, | ,, | ,, | 19·7 | 25·0 | 17·0 |
| 982 | ,, | ,, | ,, | 20·8 | 24·0 | 18·0 |
| 2431 | Reedbuck | ,, | ,, | 24·7 | 30·0 | 17·0 |
| 2359 | ,, | ,, | ,, | 22·9 | 28·0 | 20·0 |
| 2429 | ,, | ,, | ,, | 21·7 | 30·0 | 18·0 |
| 2445 | ,, | ,, | ,, | 20·7 | 27·0 | 15·0 |
| 2357 | ,, | ,, | ,, | 21·6 | 25·0 | 18·0 |
| 2429 | ,, | ,, | ,, | 22·7 | 27·0 | 18·0 |
| 2371 | Bushbuck | ,, | ,, | 19·5 | 22·0 | 16·0 |
| 2372 | ,, | ,, | ,, | 19·9 | 24·0 | 16·0 |
| 2371 | ,, | ,, | ,, | 20·8 | 24·0 | 18·0 |
| 2327 | ,, | ,, | ,, | 22·1 | 27·0 | 19·0 |
| 2371 | ,, | ,, | ,, | 20·8 | 24·0 | 19·0 |
| 2372 | ,, | ,, | ,, | 21·5 | 26·0 | 19·0 |
| 2484 | Rat | ,, | ,, | 19·4 | 27·0 | 15·0 |
| 2494 | ,, | ,, | ,, | 19·6 | 26·0 | 16·0 |
| XC | ,, | ,, | ,, | 22·4 | 30·0 | 18·0 |
| 2457 | ,, | ,, | ,, | 21·4 | 30·0 | 16·0 |
| 24 | ,, | ,, | ,, | 21·4 | 29·0 | 17·0 |
| 129 | ,, | ,, | ,, | 25·4 | 32·0 | 18·0 |
| 201 | ,, | ,, | ,, | 23·6 | 29·0 | 13·0 |
| — | ,, | ,, | ,, | 26·3 | 31·0 | 16·0 |
| | | | | 22·1 | 33·0 | 13·0 |

The average length of *Trypanosoma gambiense* in man and the other species of animals, taken from Table I, is as follows :—

Table II.

| Species of animal. | In microns. | | |
|---|---|---|---|
| | Average length. | Maximum length. | Minimum length. |
| Man | 24·3 | 33·0 | 15·0 |
| Chimpanzee | 23·4 | 33·0 | 18·0 |
| Monkey | 22·4 | 31·0 | 15·0 |
| Ox | 19·5 | 29·0 | 15·0 |
| Reedbuck | 22·4 | 30·0 | 15·0 |
| Bushbuck | 20·7 | 27·0 | 16·0 |
| Rat | 22·4 | 32·0 | 13·0 |

CHART I.—Chart giving Curve representing the Distribution, by Percentages, in respect to Length of 1000 Individuals of *Trypanosoma gambiense.*



*Breadth.*—The long and slender forms average 1·5 microns, the short and stumpy 2·5 microns.

*Shape.*—This, as stated above, is a markedly dimorphic species.

*Contents of Cell.*—The protoplasm often shows many chromatin granules in its substance.

*Nucleus.*—Resembles *Trypanosoma brucei,* in that the nucleus is oval in the long and slender, and round in the short and stumpy forms.

*Micronucleus.*—Small and round, and situated, on an average, 1·1 microns from the posterior extremity in the short and stumpy, 1·3 in the intermediate, and 1·8 in the long and slender forms.

*Undulating Membrane.*—This, as in *Trypanosoma brucei,* is well developed, and thrown into many bold folds and undulations.

*Flagellum.*—The flagellum in the long and slender and intermediate forms is free. There is no free flagellum in the short and stumpy forms.

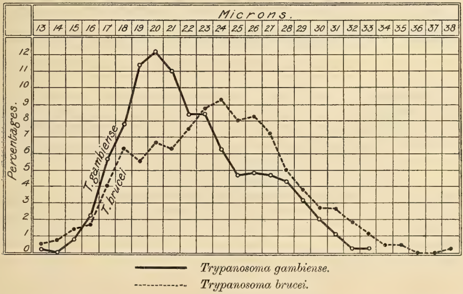
Table III.—Distribution in respect to Length of 1000 Individuals of *Trypanosoma gambiense*, Uganda strain.

| Animal. | In microns. | | | | | | | | | | | | | | | | | | | | | Average length. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | |
| Man ............ | — | — | 3 | 5 | 5 | 3 | 3 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | 17·0 |
| ,, | — | — | — | — | — | — | — | 1 | 1 | — | 3 | 3 | 1 | 1 | 2 | 5 | 1 | 2 | — | — | — | 25·8 |
| ,, | — | — | — | 1 | — | — | 3 | 1 | 2 | 1 | — | — | — | 2 | 1 | 4 | 1 | 2 | 1 | 1 | — | 25·0 |
| ,, | — | — | — | — | — | — | 1 | 1 | — | 1 | 3 | 3 | 1 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | — | 25·7 |
| ,, | — | — | — | — | — | — | — | — | 1 | 1 | — | 1 | 1 | 1 | — | 4 | 4 | 1 | 3 | 1 | 2 | 28·3 |
| Chimpanzee ... | — | — | — | — | — | 1 | 4 | 2 | 3 | 3 | 2 | 2 | — | — | 1 | 1 | 1 | — | — | — | — | 22·0 |
| ,, | — | — | — | — | — | — | 1 | — | 4 | 3 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | — | 1 | 24·6 |
| ,, | — | — | — | — | — | 3 | — | 1 | 1 | 3 | 4 | — | 2 | 1 | 2 | 1 | — | — | 1 | — | 1 | 23·7 |
| Monkey ...... | — | — | — | — | 1 | 1 | 2 | 4 | 3 | 2 | 2 | — | — | — | 1 | — | 3 | — | 1 | — | — | 22·5 |
| ,, | — | — | 1 | — | 2 | — | 4 | 2 | 1 | 1 | 4 | 1 | 1 | 1 | 1 | 1 | — | — | — | — | — | 21·5 |
| ,, | — | — | — | — | — | 1 | — | 1 | — | 1 | 1 | 5 | 3 | 2 | 3 | 2 | 1 | — | — | — | — | 24·8 |
| ,, | — | — | — | — | 1 | — | 2 | 6 | — | 2 | 2 | — | 2 | 1 | 1 | — | 2 | — | 1 | — | — | 22·8 |
| ,, | — | — | — | — | — | 4 | 4 | 2 | 1 | 1 | 4 | 2 | 2 | — | — | — | — | — | — | — | — | 21·0 |
| ,, | — | — | — | 1 | 1 | 1 | 2 | 2 | 1 | 5 | 4 | 1 | — | 2 | — | — | — | — | — | — | — | 21·4 |
| ,, | — | — | — | — | 5 | 3 | 3 | 4 | 1 | — | 2 | 1 | — | 1 | — | — | — | — | — | — | — | 19·6 |
| ,, | — | — | — | 1 | 3 | 3 | 4 | 3 | 2 | — | — | 1 | — | — | 2 | — | — | 1 | — | — | — | 20·3 |
| ,, | — | — | — | — | — | 1 | 3 | 1 | 1 | 3 | — | 2 | 2 | 1 | 2 | 3 | — | 1 | — | — | — | 23·7 |
| ,, | — | — | — | — | 1 | 1 | 3 | 1 | 2 | 2 | 1 | 3 | 2 | 1 | 1 | — | 2 | — | — | — | — | 22·7 |
| ,, | — | — | — | — | — | — | 3 | 2 | 6 | 2 | 3 | — | 1 | — | 1 | 2 | — | — | — | — | — | 22·2 |
| ,, | — | — | — | — | 1 | 2 | — | 2 | 1 | 2 | 3 | 3 | 1 | — | — | 1 | 2 | 2 | — | — | — | 23·5 |
| ,, | — | — | — | — | 2 | 4 | 3 | 6 | 2 | — | — | — | — | 2 | 1 | — | — | — | — | — | — | 20·2 |
| ,, | — | — | — | — | — | 1 | — | — | 2 | 3 | 1 | 2 | 2 | 3 | 3 | 1 | — | 1 | 1 | — | — | 24·7 |
| ,, | — | — | — | — | — | — | — | 1 | — | — | 1 | 2 | 2 | 5 | 5 | 1 | — | 3 | — | — | — | 26·2 |
| Ox ............... | — | — | — | — | 5 | 3 | 4 | 2 | 3 | 1 | 2 | — | — | — | — | — | — | — | — | — | — | 19·3 |
| ,, | — | — | — | — | 2 | 4 | 6 | 2 | 3 | 1 | — | — | — | — | — | 1 | 1 | — | — | — | — | 20·1 |
| ,, | — | — | — | 1 | 4 | 5 | 3 | 3 | 2 | 1 | 1 | — | — | — | — | — | — | — | — | — | — | 18·9 |
| ,, | — | — | — | 3 | 3 | 4 | 3 | 3 | — | 3 | — | — | — | 1 | — | — | — | — | — | — | — | 19·0 |
| ,, | — | — | 3 | 1 | 3 | 2 | 3 | 1 | 4 | — | 1 | 1 | 1 | — | — | — | — | — | — | — | — | 19·0 |
| ,, | — | — | — | — | 2 | 2 | 7 | 4 | 2 | 1 | 1 | — | 1 | — | — | — | — | — | — | — | — | 19·7 |
| ,, | — | — | — | — | — | 2 | 3 | 5 | 3 | 2 | 3 | 2 | — | — | — | — | — | — | — | — | — | 20·8 |
| Reedbuck ...... | — | — | — | — | 1 | — | 1 | 2 | 1 | 1 | 1 | — | 2 | 3 | 3 | 3 | — | 2 | — | — | — | 24·7 |
| ,, | — | — | — | — | — | — | — | 1 | 7 | 2 | 2 | 3 | 2 | 2 | — | 1 | — | — | — | — | — | 22·9 |
| ,, | — | — | — | — | — | 1 | 2 | 3 | 6 | 5 | — | — | 1 | — | — | 1 | — | 1 | — | — | — | 21·7 |
| ,, | — | — | 1 | — | 2 | — | 5 | 4 | — | 3 | 2 | — | — | 2 | 1 | — | — | — | — | — | — | 20·7 |
| ,, | — | — | — | — | — | 1 | 5 | 2 | 1 | 3 | 2 | 3 | 3 | — | — | — | — | — | — | — | — | 21·6 |
| ,, | — | — | — | — | — | 1 | — | 3 | 2 | 2 | 5 | 3 | 1 | 2 | 1 | — | — | — | — | — | — | 22·7 |
| Bushbuck...... | — | — | — | 1 | — | 4 | 4 | 6 | 4 | 1 | — | — | — | — | — | — | — | — | — | — | — | 19·5 |
| ,, | — | — | — | 2 | 2 | 2 | — | 5 | 4 | 3 | 1 | 1 | — | — | — | — | — | — | — | — | — | 19·9 |
| ,, | — | — | — | — | — | 3 | 2 | 4 | 4 | 2 | 4 | 1 | — | — | — | — | — | — | — | — | — | 20·8 |
| ,, | — | — | — | — | — | — | 3 | 3 | 3 | 1 | 4 | 4 | 1 | — | 1 | — | — | — | — | — | — | 22·1 |
| ,, | — | — | — | — | — | — | 4 | 7 | 4 | 1 | 2 | 2 | — | — | — | — | — | — | — | — | — | 20·8 |
| ,, | — | — | — | — | — | — | 3 | 4 | 5 | 3 | 1 | 1 | 2 | 1 | — | — | — | — | — | — | — | 21·5 |
| Rat ............ | — | — | 1 | 2 | 3 | 3 | 2 | 1 | 5 | 1 | — | 1 | — | — | 1 | — | — | — | — | — | — | 19·4 |
| ,, | — | — | — | 1 | 4 | 5 | 2 | 3 | 1 | — | 1 | 1 | — | 2 | — | — | — | — | — | — | — | 19·6 |
| ,, | — | — | — | — | — | 2 | 2 | 4 | 3 | 1 | — | 1 | 2 | 2 | 2 | — | — | 1 | — | — | — | 22·4 |
| ,, | — | — | — | 1 | 1 | 2 | 4 | 1 | — | 6 | 1 | — | 2 | — | — | — | 1 | 1 | — | — | — | 21·4 |
| ,, | — | — | — | — | 2 | 2 | 1 | 4 | 4 | 1 | 2 | — | 1 | 1 | 1 | — | 1 | — | — | — | — | 21·4 |
| ,, | — | — | — | — | — | 2 | — | — | 1 | 2 | 1 | — | 1 | 3 | 3 | 6 | — | — | — | 1 | — | 25·4 |
| ,, | 1 | — | — | — | — | — | — | 1 | 3 | — | 5 | 3 | 2 | 1 | 1 | 1 | 2 | — | — | — | — | 23·6 |
| ,, | — | — | — | 1 | — | — | — | — | — | 2 | 1 | 1 | — | 2 | 4 | 3 | 5 | — | 1 | — | — | 26·3 |
| Total......... | 1 | — | 9 | 21 | 56 | 79 | 114 | 122 | 110 | 85 | 85 | 61 | 47 | 49 | 47 | 44 | 31 | 20 | 11 | 4 | 4 | |
| Percentages | 0·1 | — | 0·9 | 2·1 | 5·6 | 7·9 | 11·4 | 12·2 | 11·0 | 8·5 | 8·5 | 6·1 | 4·7 | 4·9 | 4·7 | 4·4 | 3·1 | 2·0 | 1·1 | 0·4 | 0·4 | |

## COMPARISON OF TRYPANOSOMA GAMBIENSE WITH TRYPANOSOMA BRUCEI.

On comparing the coloured plate of *Trypanosoma gambiense,* given at the end of this paper, with that of *Trypanosoma brucei,*[*] it will be apparent that these two species of trypanosomes resemble each other very closely. There are the same long and slender, intermediate, and short and stumpy forms in both. The micronucleus is small and round, the nucleus oval or round, and the undulating membrane well developed. It may be concluded, then, that it is impossible to separate these two species by shape alone.

CHART II.—Chart giving Curves representing the Distribution, by Percentages, in respect to Length of 1000 Individuals of *Trypanosoma gambiense*, Uganda, and *Trypanosoma brucei.*



On comparing the curves representing the distribution by percentages in respect to length of 1000 individuals of each species, some slight difference can be made out. It is seen that *Trypanosoma gambiense* lies more to the short end of the curve than *Trypanosoma brucei.* There are more non-flagellated forms in *Trypanosoma gambiense* than in *Trypanosoma brucei:* 38 per cent. in the former, 26 per cent. in the latter. It is doubtful, however, if this difference in the curve would always appear.

In the same way, if the 1000 *Trypanosoma gambiense* are divided by length into short and stumpy (13 to 21 microns), intermediate (22 to 24 microns), and long and slender (25 microns and upwards), as was done in the case of *Trypanosoma brucei*, the following is the result :—

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[*] 'Roy. Soc. Proc.,' B, vol. 83, Plate 2.

| | Short and stumpy. | Intermediate. | Long and slender. |
|---|---|---|---|
| | per cent. | per cent. | per cent. |
| *Trypanosoma gambiense* ...... | 51·2 | 23·1 | 25·7 |
| *Trypanosoma brucei* ............ | 32·8 | 25·5 | 41·7 |

This shows the percentage of the intermediate to be much the same in the two species, whereas *Trypanosoma gambiense* is richer in short forms and poorer in long than *Trypanosoma brucei.*

Whether these slight differences are fundamental or only accidental it is impossible at present to say, but enough has been written to show that *Trypanosoma gambiense* and *Trypanosoma brucei* approach each other very closely in size and shape.

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## *The Behaviour of the Infusorian Micronucleus in Regeneration.*

By Kenneth R. Lewin, B.A., Senior Scholar and Coutts Trotter
Student of Trinity College, Cambridge.

(Communicated by Prof. J. S. Gardiner, F.R.S. Received September 15,—Read November 2, 1911.)

The older experimenters on Infusoria have left very few records of the behaviour of the micronucleus in regeneration.

Balbiani ('91), in discussing the subject, declares that the presence of a micronucleus is not essential to regeneration, since he had examined certain regenerated merozoa* of *Frontonia, Prorodon, Trachelius,* and *Stentor* without finding a micronucleus even with the aid of reagents. He also cut conjugating couples of *Stentor* at a time when the old meganucleus had started to degenerate, and found that, unless a new meganucleus were formed, none of the merozoa could regenerate. He concluded that the micronucleus has no influence on regeneration.

Stevens ('03) performed some experiments on *Licnophora,* and found that regeneration occurred only in the micronucleate piece ; but as no regeneration took place if more than three-quarters of the oral disc were removed, in

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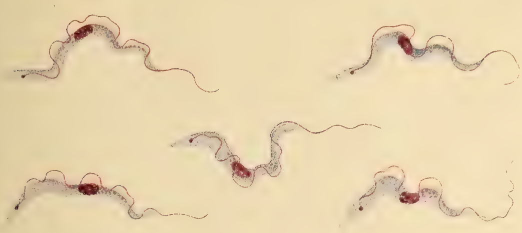
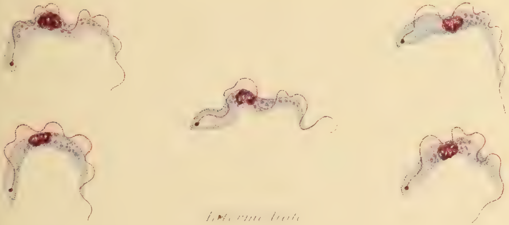
* The word "merozoite" being well established in Sporozoan literature, I propose to use the term "merozoon" suggested by Johnson ('93) for a cut fragment of an Infusorian.

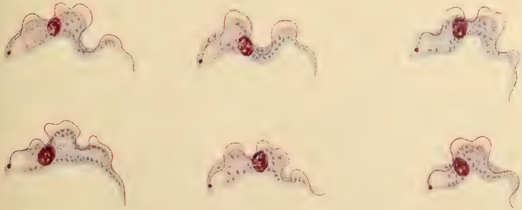
## *Trypanosoma gambiense.*



*Long & Slender.*



*Intermediate.*



*Short & Stumpy.*

M. E. Bruce, del.

spite of the continued presence of the micronucleus, the evidence only indicates the importance of leaving part of the oral disc, and leaves the question of the possible influence of the micronucleus quite undecided, as, indeed, the authoress states.

Of late, however, Calkins ('11) has given evidence which goes some way to show that in *Uronychia* the micronucleus, though not always necessary to regeneration, exerts an influence on the process. Cutting this hypotrich when division had taken place less than an hour previously, he found that in 2 cases out of 13 neither merozoon regenerated, and that in the remaining 11 one merozoon only in each case produced a normal animal, this being invariably the one with the single micronucleus characteristic of the species.

Also, in cells cut between 8 and 16 hours after division (the period between two divisions being 36 hours), out of nine recorded experiments in which both merozoa were preserved, seven showed a regeneration of one, and that the micronucleate, merozoon only. In the other two, both pieces regenerated.

These experiments favour the view that regeneration cannot occur in the absence of the micronucleus, unless the cell operated upon is at least from 8 to 16 hours old. This conclusion must, I think, be accepted with great caution, especially as but little information is vouchsafed as to how the highly differentiated soma of *Uronychia* was divided. In the cases figured there is only one (fig. 6) in which the micronucleus fell to the portion of the anterior merozoon.

It must also be pointed out that Calkins does not consider the possibility of the micronucleus being reformed from the meganucleus in the cases where regeneration occurs. It is impossible to determine the presence of the organella in the life of the animal, and from Calkins' statements it is not clear how he found out which merozoon had received the micronucleus. In only two cases does he expressly say that the unregenerate was killed and stained (Calkins' Expts. 28 and 34, and his figs. 7 and 4B).

His fig. 4B shows a trilobed meganucleus, and a spherical fragment which from the sketch appears as likely to be a micronucleus as to be a detached piece of the meganucleus. In an ordinary stained preparation of the animal there is but a slight difference in texture to be distinguished between the two nuclei. I reproduce Calkins' fig. 4B in my fig. A, together with a second drawing (X) of the unregenerate, with the doubtful body represented as a micronucleus; Calkins gives no proof of its meganuclear nature. By the supposition that the micronucleus could be re-formed in regeneration, all the results reported by him could be explained, at least, as regards the supposed influence of the micronucleus.

This caution in accepting Calkins' conclusions does not necessarily imply a belief that they are incorrect. There lies, however, at the base of his reasoning the tacit assumption that the micronucleus is never regenerated. Further, he does not establish clearly that the micronucleus was the *only* organella in the possession of which the regenerates differed from the merozoa which were unable to recover the normal form.

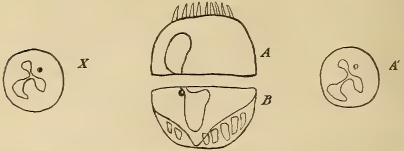


Fig. A.—Experiment on *Uronychia transfuga.* *A*, *B*, and *A'* copied from Calkins. *X* modified from *A'*.

Concerning the regeneration of the micronucleus from the meganucleus the literature furnishes only the unsatisfactory note of Le Dantec ('97) which states that in experiments on various monomicronucleate ciliates, not further particularised, a micronucleus was found later in merozoa which had been rendered amicronucleate by the operation. In no later paper does the French author return to the subject, so a degree of scepticism is natural.

I have shown (Lewin '11) that in *Paramecium* there occurs no regeneration of the micronucleus in ordinary conditions and that the presence of this organella is in no way necessary for the continuance of normal asexual life, with multiplication by fission.

### *Observations on* Stylonychia mytilus.

These observations are only the beginning of the complete investigation that the subject demands, and are the more imperfect because of the difficulty I found in keeping healthy cultures in Naples. The excellent drinking water there is fatal to *Stylonychia* in a few hours; boiled and aërated it varies in its effects in a manner for which I am totally unable to account.

One stock culture alone, out of many laid down, has flourished to any extent, and most of my later experiments on individuals from this have been entirely vitiated by the discovery of frequent nuclear irregularities.

*Methods.*—Single animals were placed in a drop of the mucilage of "Alga caragheen" on a slide thinly coated with paraffin. They were cut with a special cutting apparatus designed for use with the system obj. A (Leitz) and oc. 2. The merozoa were isolated in nutritive solution on hollow-ground slides in a moist chamber. Fresh or one-day-old hay infusion was used as culture fluid, with or usually without the addition of a few *Colpidia*. *Stylonychia* thrives quite well on a diet of bacteria and the nuclear relations are not obscured in preparations by the presence of the nuclei of ingested prey.

Nuclear phenomena were examined in glycerine mounts of animals fixed and stained by Schneider's acetocarmine, which gives a perfectly clear picture of the nuclei.

In all the experiments I am about to describe, the hind end of the animal was removed by a transverse cut. Only in a few cases did the cut pass between the two members of the meganucleus; the most interesting results were obtained when the nuclear apparatus was left untouched.

These were the earlier experiments; the removal of the anterior end was attempted later, when it was found that the stock culture was in a very heterogeneous condition as regards the nuclear relations of its individuals, and the results of these cuttings will not be dealt with. As far as could be ascertained, they are in full agreement with those which follow.

The result I shall establish is briefly this:—In the regeneration which follows merotomy, multiplication of micronuclei may occur, and *this increase may cause the regenerated individual to have more micronuclei than the number typical of the species or race.*

*Stylonychia mytilus* (O.F.M.) is a hypotrichous ciliate of considerable differentiation of parts (see fig. B).

The nuclear apparatus consists of a bimembered meganucleus, to the left of each node of which lies a micronucleus. There is a variety with four micronuclei, but with this I have had nothing to do. There is a fine connection between the members of the meganucleus, but it is usually invisible in stained and cleared preparations.

I shall use the following formula to express the nuclear relations:—

$$\text{M}, am : \text{M}, bm.$$

M stands for a member of the meganucleus, and $m$ for a micronucleus, $a$ being the number lying by the anterior M, and $b$ the number by the posterior M.

As is implied thus, the anterior M is written first. The normal condition of the animals with which I worked was

$$\text{M}, 1m : \text{M}, 1m.$$

Or written more simply,

$$\text{M}, m : \text{M}, m.$$

The irregularities which cropped up in the stock culture were the occurrence of animals of the types :—

M, 2$m$ : M, $m$, and M, $m$ : M, 2$m$.

*Cases of Equal Section.*—In these I include all cases in which the plane of section passed between the two members of the meganucleus. Each of the merozoites thus obtained is capable of regeneration and subsequent division.

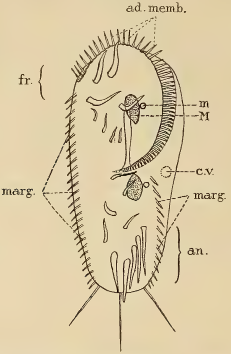


Fig. B.—*Stylonychia mytilus* (semi-diagrammatic). Ventral view. × 300.

*ad. memb.*, adoral membranellæ. *M*, mega-, *m*, micronucleus.
*fr.*, frontal cirri. *c.v.*, contractile vacuole.
*an.*, anal cirri. *marg.*, marginal cirri.

The initial condition is M, $m$, but regeneration includes segmentation of the M into two members, and the division of $m$. The normal relation, M, $m$ : M, $m$ is thus attained.

The division of the micronucleus might, from a consideration of this case alone, be regarded as part of the mechanism of organic regulation, since its effect is to restore the normal proportions.

*Cases of Removal of a Plasmatic Portion from the Posterior End.*—In none of these was the nuclear apparatus disturbed by the section.

(1) Merozoon killed 4½ hours after section.

M, $m$ : M, $m$*.

The posterior micronucleus *m** was in an early stage of division. (Fig. 1.)

(2) Merozoon killed $3\frac{3}{4}$ hours after section.

M, *m* : M, *mm*.

The posterior micronucleus was in division (*mm*). (Fig. 2.)

(3) Merozoon killed 3 hours after section.

M, *mm*† : M, *m*†.

The micronuclei marked *m*† were in process of reconstruction after division. (Fig. 3.)

(4) Merozoon killed $3\frac{1}{4}$ hours after section.

M, *mm*† : M, *m*†.

Reconstruction of *m*† had gone a little further than in (3). (Fig. 4.)

(5) Merozoon killed $18\frac{1}{2}$ hours after section.

M, 2*m* : M, *m*. (Fig. 5.)

Most probably this is a case where the posterior micronucleus had divided. Fig. 4 shows that in that case two micronuclei would be found anteriorly.

There is a possibility that this is merely one of the abnormalities which became frequent later, and that the animal was so constituted before the operation.

(6) Merozoon had divided 27 hours after section. Both daughters were killed. Micronuclear division had presumably taken place during regeneration, and the six micronuclei found altogether in the two animals were formed by the division of the three present after regeneration was complete. I do not attempt to explain in detail the irregular distribution to the daughters, or the unusual positions of some of the micronuclei in the cell.

It is not known which of the individuals arose from the anterior part of the merozoon. (Figs. 6 and 7.)

2M, 4*m*, and 2M, 2*m*.

(7) Had divided 46 hours after section.

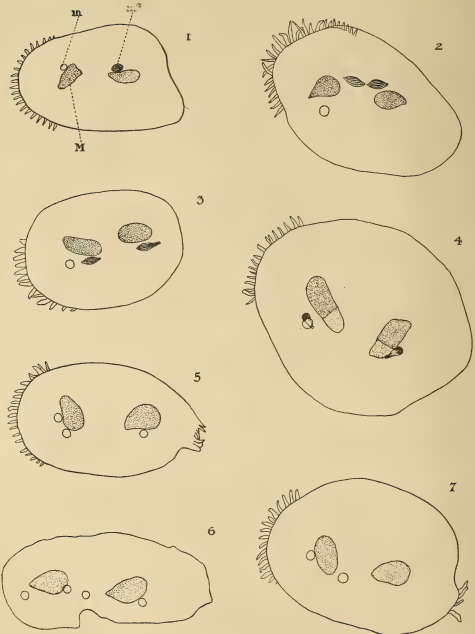
Each daughter had divided 24 hours later, and a sample of each was killed. (Figs. 8 and 9.)

M, *m* : M, 2*m*, and M, 2*m* : M. *m*.

By the next day each had divided again, and samples were again killed, the nuclear relations in both cases being:—

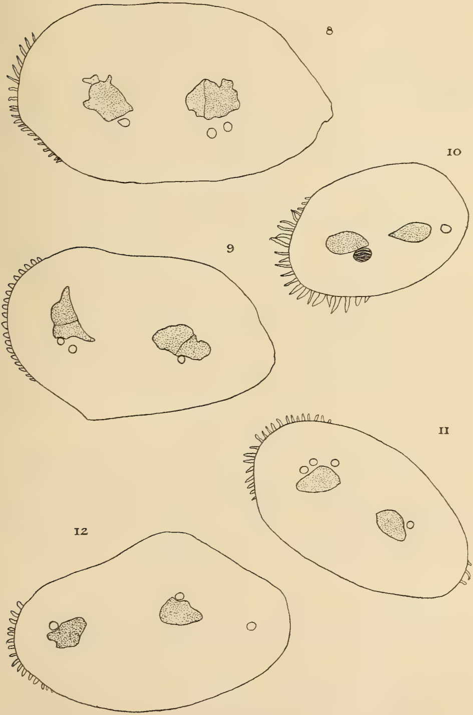
M, *m* : M, 2*m*.

An accident caused the loss of both survivors.



FIGS. 1—7.

The figures were drawn from animals fixed and stained with Schneider's acetocarmine and mounted in glycerine. This method does not lend itself to the preservation of cytoplasmatic detail, but in practically every case the adoral membranellæ could be distinguished, and are represented to mark the anterior end of the animal. The outlines were sketched with the aid of a camera lucida, and the magnification was 300 (Zeiss apochr., obj. 3 mm., oc. 4).



Figs. 8—12.

It is quite possible that every division produced daughters respectively: —

M, $2m$ : M, $m$, and M, $m$ : M, $2m$.

The experiments so far described make a series which proves definitely that the regenerative processes involve in some cases a division of the micronucleus.

When a *Stylonychia* is cut equally the mitosis always here associated with regeneration restores the normal number of micronuclei. In these experiments, too, there is a reorganisation of the meganucleus, which does not occur when a purely plasmatic portion of the animal is removed.

In the numbered series of experiments, on the other hand, the mitosis is no regulative action, but results in the regenerate having an abnormally large number of micronuclei.

More than 50 per cent. of the experiments in plasmatic removal gave negative results with regard to nuclear division, *i.e.*, the fully regenerated merozoa had the normal nuclear relation, M, $m$ : M, $m$.

Two cases must now be given in which there is indicated a division of the *anterior* micronucleus, *i.e.*, that farther from the plane of section. It is possible that this occurred more often, but in view of the heterogeneity of my material during the later experiments I cannot be certain of this.

(8) Merozoon killed $2\frac{3}{4}$ hours after section.

M, $m^*$ : M, $m$.

$m^*$ is in an early stage of division. (Fig. 10.)

(9) 1 hour after section the frontal cirri could not be seen. The animal was in continual rotation. Between 3 and 5 hours after section the adoral membranellæ were withdrawn. 7 hours after section the adoral membranellæ were regenerated. No anal cirri seen. $22\frac{1}{2}$ hours after section the ciliation was normal, but the posterior end of the regenerate was rather pointed. Staining revealed the fact that the anterior micronucleus had divided:—

M, $m$ : M, $2m$.

The infusorian was rather more transparent than is usual, and careful observation before cutting had made me fairly confident that the original relations were normal:—

M, $m$ : M, $m$.

That the posterior micronucleus did not divide is in no way surprising, for as is mentioned above, in more than 50 per cent. of the experiments there was no mitosis caused.

Two more experiments may be quoted, in both of which it is probable that the posterior micronucleus divided.

(10) Merozoon killed 4¼ hours after section.

M, 3*m* : M, *m*.

I have never found in the stock culture an individual with so uneven a distribution of micronuclei, so it seems probable that the cut animal had nuclei :—

M, 2*m* : M, *m*.

Division of the posterior micronucleus would then give the relation observed. (Fig. 11.)

(11) Merozoon killed 22 hours after section.

In the hinder half were two micronuclei, one in the usual position close to the meganucleus, and one right at the posterior border of the body.

M, *m* : M, *mm*. (Fig. 12.)

I interpret this as a case where one daughter nucleus was carried at the end of the spindle to the posterior margin of the body, and not anteriorly to the side of the front member of the meganucleus.

*Discussion.*—It is certain that division of the micronucleus can be caused to occur during the processes of regeneration which follow operation.

In the majority of cases in which mitosis occurred, it was the micronucleus near the cut surface which divided, indicating that division is caused by local formative processes rather than by the general condition of the cell. The few puzzling cases in which the anterior micronucleus divided, have a light thrown on them by experiment (9). Here the absorption and subsequent regeneration of the adoral membranellæ made the anterior region a locality of constructive activity, whilst the regeneration of the hind end proceeded with abnormal slowness. The absorption and subsequent regeneration of the adoral membranellæ do not usually occur. In the great majority of cases at no time is the animal without these organellæ; if any reorganisation occurs it goes on by imperceptible stages.

Now in more than 50 per cent. of the experiments there was no nuclear increase, so regeneration *can* go on without causing the micronucleus to divide. In these cases either the micronucleus was in a state different from that obtaining in the successful experiments, or the conditions in the cell were not such as to cause mitosis.

Further research will show if there be a connection between the age of the individual and the readiness of the micronucleus to divide. It may well be, also, that the stimulus to division has to surmount some threshold before it becomes effective. Before speaking of this, however, it is necessary to summarise the evidence for regarding the micronucleus as an organella of some independence during the asexual period, living in the plasma as it were in a nutritive solution, and pricked on to division by some definite change in its environment:—

(*a*) The cell can live very well without the micronucleus, in the case of *Paramecium* at least. (Lewin '11.)

(*b*) The division of the micronucleus can occur independently of cell and of meganuclear division, *e.g.*, in depression (Popoff '09), in regeneration and in conjugation.

(*c*) The behaviour of the micronucleus in regeneration (in *Stylonychia*) is not of the nature of a regulation; in fact, its effect may be increase of the micronucleus above the normal number.

These considerations justify, I think, the heuristic conception of the micronucleus as living independently during the asexual cycle with the cell as its environment; it is independent in so far as it can lie outside that narrower individuality of the organism which is jealously preserved by the processes of organic regulation.

Whether this view can be extended to the micronucleus at the time of conjugation remains to be seen; very possibly at this epoch in infusorian life the organella in question loses its independence and merges in the regulated individuality of the animal. Otherwise it is not at once clear how the great regularity of nuclear organisation after conjugation is brought about. I may point out that the measure of independence here conceptually accorded to the micronucleus in no way precludes the possibility of its influencing and being influenced by the cell in which it lies, just as infusoria in a culture fluid influence it and it them.

In this way I regard the micronucleus as being ripe for division, if not throughout the whole of its resting phase, at least long before the cell is ready for fission. There needs only to arise the appropriate stimulus, *i.e.*, the proper chemical or electrical condition of the circumambient plasma, and mitosis will occur.

At division, and in regeneration, this stimulus is associated with local formative activity; local, since in regeneration it is the nucleus near the place of active formation of new parts which divides.

In the artificial depression induced by Popoff in *Stylonychia* the stimulus, if it be the same, is associated with a disturbance of the normal metabolism, such as occurs in "natural" depression.

At conjugation the independent divisions of the micronucleus are of a special type, owing either to a difference in the stimulus or to a change in the nucleus itself. I cannot agree with Popoff when he compares the divisions he observed with the micronuclear multiplication at conjugation, for in his figures the mitoses appear to be quite of the ordinary type.*

Resuming the discussion of the experimental results, it is now clear what I mean by the stimulus having perhaps to surmount some threshold before causing mitosis. It can easily be imagined that the grade of constructive activity must reach a certain degree of steepness before it or some concomitant can start the micronucleus dividing. Hence, if it be assumed that the grade of activity can be measured by the whole time taken by regeneration, when the process goes on slowly, no mitosis would be expected.

I suppose that the cases in which no mitosis occurred differed from the successful experiments either in the age of the micronucleus, or in the intensity of the constructive processes. Both factors may work together, but that the latter is concerned is indicated in a measure by experiment (9). Here there was the usual regeneration of the cirri and of the hind end of the body taking place more slowly than usual. (Regeneration is normally accomplished in from three to five hours, and after seven the posterior ciliation was not complete in this case.) The posterior micronucleus did not divide.

Anteriorly, the adoral membranellæ, withdrawn between the third and the fifth hour after section, were replaced by the seventh hour, indicating considerable formative intensity. The anterior micronucleus *did* divide.

One factor in the organisation of *Stylonychia* remains to be mentioned, viz., the unknown influences which determine the situation of the micronuclei in the cell. Normally, each lies by a member of the meganucleus. Of the nature of the forces which bring this about nothing is known.

After the increase in the number of micronuclei accompanying regeneration, the supernumerary nucleus usually lies by the side of the meganuclear element other than that by which its sister is found. The normal spindle-length in mitosis in *Stylonychia* spans the distance between the two members of the meganucleus. This regularity does not always obtain, as can be seen from figs. 6 and 7. The failure of the forces to order the arrangement of the micronuclei in the usual way is probably the cause of the unequal distribution between the sister animals.

Fig. 2 also indicates an irregularity, the anterior micronucleus lying on one side of the meganucleus, and the posterior (in division) on the other, a most unusual thing. Fig. 12 strongly suggests that the unknown forces have utterly failed to direct the division of the hind micronucleus, with the result that one product has been carried to the very posterior end of the cell.

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* In *Paramecium* (in which Popoff observed similar phenomena) at least one would expect to see the micronucleus in the characteristic "crescent" stage which precedes the formation of the spindle for the first division in conjugation. Popoff figures several mitoses, but does not refer to this stage.

These phenomena are to be compared with those exhibited by the micronucleus of *Paramecium*. Normally lying in a notch in the side of the meganucleus, the organella moves out, or is swept away, into the endoplasm on occasions when the animal is starving or in depression. Here is shown the same stereotyped position of the micronucleus, and the same occasional failure of the forces concerned to maintain it.

The experiments recorded above were performed at the Stazione Zoologica, in Naples, during my tenure of the Cambridge University table there. To the officials of the station I wish to express my thanks for many kindnesses.

I am indebted as Coutts Trotter Student to the authorities of Trinity College for permission to hold the studentship at Naples.

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# *The Refractive Indices of the Eye Media of some Australian Animals.*

By Judah Leon Jona, D.Sc., M.B., B.S.

(Communicated by Prof. J. N. Langley, F.R.S. Received August 23,—
Read November 16, 1911.)

(From the Physiological Department of the University of Melbourne.)

While carrying out an investigation on the osmotic pressure of the blood and body fluids of various Australian animals I was desirous of supplementing the data thus acquired with estimations of other physical characters. The refractive indices of the eye fluids presented some interesting features, and I give here in tabular form the results obtained.

The instrument employed was the Abbé refractometer. The outer layers of the crystalline lens were sufficiently fluid to be spread out as a refracting layer on the prism.

| | $\mu$ of aqueous humour. | $\mu$ of vitreous humour. | $\mu$ of lens (outer layers). | Temp., ° C. |
|---|---|---|---|---|
| *Land Mammals*— | | | | |
| Rabbit | 1·336 | — | 1·43 | 10·3 |
| Echidna (A) | 1·336 | 1·338 | — | 16·5 |
| (B) | 1·337 | — | — | 15·5 |
| *Land Reptile*— | | | | |
| *Emydura macquaria* | 1·3357 | 1·3362 | — | 15—16 |
| *Amphibia*— | | | | |
| *Hyla aurea* | 1·3355 | — | 1·4675 | 11·7 |
| ***Fish***— | | | | |
| Teleosts— | | | | |
| Marine— | | | | |
| Barracouta (*Thyrsites atun*) (A) | 1·3353 | 1·3354 | 1·433 | 12 |
| (B) | 1·3353 | 1·3354 | — | 12 |
| Fresh Water— | | | | |
| Murray Cod (*Oligorus Macquariensis*) (A) | 1·3361 | | | |
| (B) | 1·3362 | — | 1·43 | 12 |
| Ascidian | 1·3524 | 1·3772 | 1·46 | |
| *Tap Water*— | | | | |
| Yan Yean Reservoir | 1·3336 | | | |
| *Sea Water*— | | | | |
| From Hobson's Bay | 1·3404 | | | |

In connection with these results it is of interest to note that right through the Vertebrates from Mammals down to Teleostean fishes, marine and fresh water, the refractive indices of the aqueous humour, vitreous and lens (outer layers) were about the same in each class of animal, being approximately 1·336 for aqueous, 1·337 for vitreous, and 1·46 for lens. Determinations of the $\Delta$ of the aqueous humour of various animals show that this is approximately the same as the $\Delta$ of blood serum of these animals (Portier (1), Steindorff (2) ), and thus varies in the different classes of animals.

Moreover, on comparing the refractive index of the aqueous humour of the sea-water Teleost (Barracouta) (1·335) with the refractive index of the sea water (1·340), it will be seen that we have here a unique phenomenon, virtually the tendency to the development of a "negative eye," but, of course, the almost spherical lens of refractive index about 1·46 will correct any tendency in this direction.

My thanks are due to Professor W. A. Osborne for his kind assistance and encouragement.

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## *Note on the Iridescent Colours of Birds and Insects.*

By A. Mallock, F.R.S.

[This paper is published in Series A, No. 582 (vol. A 85, pp. 598—605).]

# *Ventilation of the Lung during Chloroform Narcosis.*

By G. A. Buckmaster and J. A. Gardner.

(Communicated by Dr. A. D. Waller, F.R.S. Received August 19,—
Read November 16, 1911.

(From the Physiological Laboratory, South Kensington, University of London.)

In the report of the Chloroform Committee of the British Medical Association for 1910, the view is expressed that during chloroform narcosis the blood retains unimpaired up to the time of death its normal capacity of absorbing oxygen, and that if the amount of this gas diminishes in the blood, the decrease is solely due to the slowing of the respiration. This opinion is based on experiments made by J. Tissot. These indicate that when the respiration stops in an asphyxia induced by long anæsthetisation, 100 c.c. of the arterial blood of the dog may contain as little as 0·78 c.c. and 2·83 c.c. of oxygen, although samples taken at intervals during anæsthesia show only a slight fall in oxygen content, so long as the ventilation of the lung remained normal. In our experiments on the composition of the blood-gases during chloroform anæsthesia,* examination of the tracings of the respiratory movements during continuous inhalation of chloroform gave no indication that the progressive diminution in the amount of oxy-hæmoglobin as anæsthesia continued could be attributed to any slowing of the respiration, and none of our tracings showed any marked alteration in the frequency or amplitude of the respiratory movements. We were, therefore, unable to agree with the views expressed by Tissot. All our tracings were taken by means of a tambour applied to the chest wall in the usual way. Observations on the respiratory movements made by other observers were, as far as we can gather, made in a similar manner. It is obvious that such records afford no information which will enable an opinion to be formed with regard to the lung ventilation which is capable of precise interpretation. In order to ascertain more definitely whether the diminution in the amount of oxy-hæmoglobin in chloroform narcosis was due to changes in the type or depth of respiration, or whether it was due to the direct interference by the chloroform with the function of transporting oxygen which the red corpuscles possess, we determined to investigate the pulmonary ventilation during anæsthesia by means of a plethysmograph.

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* 'Journ. Physiol.,' Nov. 9, 1910, vol. 41, p. 246.

*Description of Plethysmograph.*

The instrument consisted of a rectangular glass box, fitting into a groove channelled in a thick slate slab forming the floor of the chamber. The box was of such a size as to comfortably hold a cat. In order to make it airtight, the groove was filled up with a stiff mixture of vaseline and beeswax. The dimensions of the box were as follows: length 67 cm., breadth 32 cm., depth 17 cm. Four holes were bored through the slate bed near the corners of the box. These were fitted with rubber corks, through which passed glass tubes of wide bore. The two holes at one end were connected with one another by a Chauveau's valve apparatus. Through a third hole, a wide tube led to a recording gasometer, made of aluminium, similar to that used by Haldane and Priestley.* The drum of the gasometer was 8 cm. high, and 6·2 cm. diameter. The gasometer, carrying a light adjustable recording lever, rose and fell in a bath of paraffin oil, which appeared to possess advantages over water. The inertia of this part of the apparatus was inappreciable. The fourth hole was connected with a bottle into which a burette fitted for purposes of graduation, and this was carried out and tested in the manner described in the paper just quoted.

The dimensions of the gasometer given were found to be most suitable for cats. In investigating respiratory ventilation, it is obviously important to avoid as far as possible breathing through long tubes and systems of valves. To minimise these disadvantages, the mixture of chloroform and air was passed through the Chauveau's valves at a slight positive pressure, and the percentage composition kept constant by the use of Waller's chloroform balance.† The animal actually respired through a cannula connecting the trachea with the central tube of the Chauveau's valve apparatus. This tube was as wide and short as possible, the length in every case being less than the distance of the tracheal opening from the mouth. It was found that expansion of air, owing to rise of temperature, caused no trouble, as during the time taken to seal up the box the temperature had become constant.

The general mode of procedure was as follows:—The animal was anæsthetised with nitrous oxide, and a cannula quickly placed in the trachea. The cannula was fitted on to the Chauveau valves, the glass box placed on the slate slab, and the whole chamber made airtight. When the animal recovered from nitrous oxide, chloroform of known percentage was administered by Waller's balance. In those experiments where a knowledge of the gas-content of the blood was required, the animal received 3 c.c. of strong hirudin solution in 1-per-cent. sodium sulphate through the femoral vein, and a cannula was tied into the carotid artery at the beginning of the experiment while it was under nitrous oxide. The animal was placed in the box, and its lung-ventilation determined for a few minutes. The glass box was rapidly lifted off the slate and a sample of blood taken. The box was then replaced and the respiration again recorded.

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* 'Journ. Physiol.,' 1905, vol. 32, p. 486.

† 'Journ. Physiol.,' Feb. 22, 1908, vol. 37.

In order to ascertain the precise effect of the inhalation of chloroform and ether on the pulmonary ventilation, it would have been desirable to determine the normal respiratory ventilation during rest. This obviously could not be determined by means of our apparatus, as an operation on the unanæsthetised animal would have been necessary; further, it was impossible to use the apparatus in the way described for human beings in Haldane and Priestley's paper, for such an animal as a cat. We thought that comparisons of the ventilation under chloroform with that during recovery from a low percentage of some anæsthetic such as nitrous oxide, the effect of which rapidly passes off and which is rapidly eliminated, would give data sufficiently reliable for our purpose. Another factor which we are unable to take into account is the question of the dead-space. This will no doubt vary in different animals, and, *cæteris paribus,* the larger the dead-space in any particular animal, the more air must it breathe in order to maintain a given alveolar ventilation. We can find no data as to the dead-space in cats, and the only satisfactory way of determining the volume of the dead-space would have been by preparing a number of plaster casts of the trachea and bronchi, such as Loewy* made when estimating the dead-space of the respiratory passages in man. We attempted to get over this difficulty by using as far as possible animals of similar size. In this paper we give the results of the effect of inhalation of chloroform and ether on the apparent total ventilation of the lung, regarded as the product of the average depth of respiration measured in cubic centimetres at 37° C., moist, and the average frequency per minute.

From a large number of records taken with varying percentages of chloroform and varying respiratory states of the animal prior to the anæsthetic, we give a selected number to illustrate the more important points which have been noticed during our experiments.

### *Effect of Different Percentages of Chloroform on the Lung Ventilation.*

In former papers we have shown that a definite danger point in chloroform anæsthesia may occur during the first few minutes, and this danger point depends essentially on two factors: firstly, the percentage of chloroform which is being inhaled, and, secondly, upon the rate and depth of the respiration prior to anæsthetisation. When the hyperpnœa is marked, or the percentage of chloroform inhaled is high, the percentage of chloroform in the blood rises with great rapidity to a maximum value, and the activity of the respiratory centre is depressed; this may occur to such an extent that the animal ceases to respire with the diaphragm always in the state of rest. This is illustrated by the following tracings of six experiments selected from a greater number (figs. 1 to 6):—

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* 'Pflüger's Archiv,' 1894, vol. 58, p. 416.

*Experiment* 1.—Effect of 1·5 per cent. chloroform after deep respirations (fig. 1):—

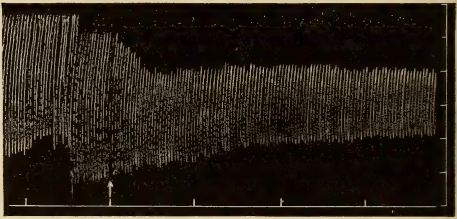


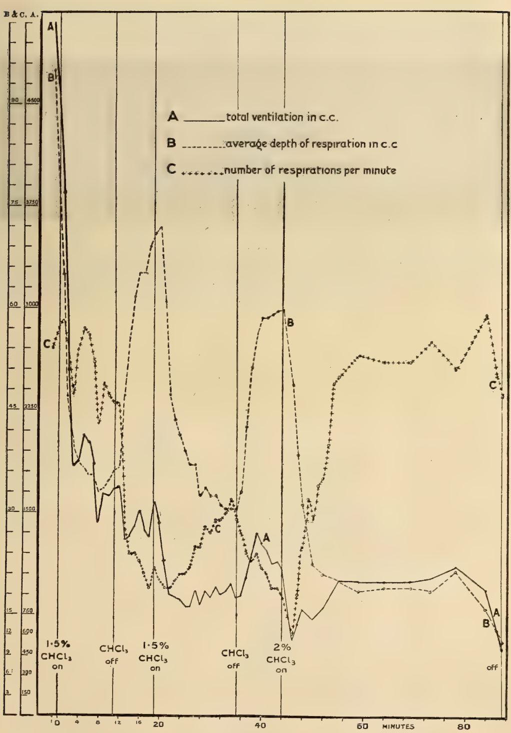
FIG. 1.

Depth of respiration, 1 division = 21·74 c.c.; time intervals, 30 secs; ↑, chloroform on.

Table I.—Measurements of Tracing in Fig. 1.

| Intervals of time, in minutes. | Anæsthetic. | No. of respirations per minute. | Average depth of respiration, in c.c. | Lung ventilation, in c.c. |
|---|---|---|---|---|
| 1 | 0 | 54 | 94·79 | 5119 |
| 0·5 | 1·5 per cent. $CHCl_3$ | 32 | 75·67 | 2421 } 3851 |
| 0·5 | ,, | 26 | 55·0 | 1430 |
| 1 | ,, | 54 | 47·19 | 2548 |

The remaining data of this experiment are given in Curve I, which shows the effect of chloroform (1·5 per cent.) for 11 minutes, recovery for 8 minutes, re-chloroforming with 1·5 per cent. for 16 minutes, recovery for 9 minutes, and, finally, re-chloroforming again with 2 per cent., death resulting in 43 minutes, in the 87th minute after the commencement of the experiment.


B & C.
A.
90
4500
75
3750
60
3000
45
2250
30
1500
15
750
12
600
9
450
6
300
3
150
A total ventilation in c.c.
B average depth of respiration in c.c
C number of respirations per minute
A
B
C
1·5% CHCl3 on
CHCl3 off
1·5% CHCl3 on
CHCl3 off
2% CHCl3 on
off
0
4
8
12
16
20
40
60
MINUTES
80

CURVE I.

*Experiment* 2.—Effect of moderate ventilation of the lung prior to the administration of 2 per cent. chloroform (fig. 2):—

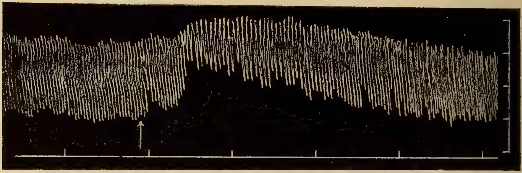


Fig. 2.

Depth of respiration, 1 division = 14·29 c.c.; time interval, 30 secs.; ↑, 2 per cent. chloroform on.

Table II.—Measurements of Tracing in Fig. 2.

| Intervals of time, in minutes. | Anæsthetic. | No. of respirations per minute. | Average depth of respiration. | Lung ventilation, in c.c. | |
|---|---|---|---|---|---|
| 1 | 0 | 55 | 48·28 | 2656 | |
| 0·5 | 2 per cent. $CHCl_3$ | 24 | 26·86 | 645 | 1161 |
| 0·5 | ,, | 19 | 27·14 | 516 | |
| 0·5 | ,, | 26 | 30·71 | 798 | 1755 |
| 0·5 | ,, | 33 | 29·0 | 957 | |
| 1 | ,, | 64 | 26·14 | 1699 | |

*Experiment* 3.—This shows the initial danger-point in chloroform anæsthesia, an effect of moderate rate with deep respiration prior to the administration of 2 per cent. chloroform (fig. 3):—

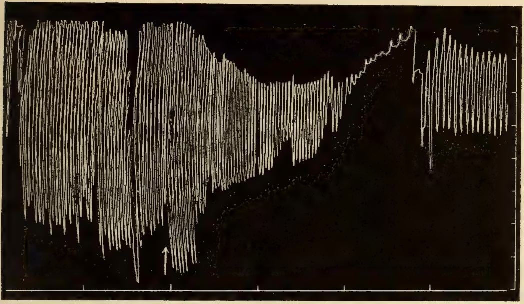


Fig. 3.

Depth of respiration, 1 division = 13·7 c.c.; time intervals, 30 secs.; ↑, 2 per cent. chloroform on.

The whole data of this experiment are given in Table III.

Table III.

| Intervals of time during which respiration was counted, in minutes. | Anæsthetic. | Frequency of respiration. | Average depth of respiration. | Total ventilation, in c.c. | Remarks. |
|---|---|---|---|---|---|
| 0·5 | Interval before $CHCl_3$, recovery from $N_2O$ | 22 | 85·35 | 1878 | |
| 0·5 | $CHCl_3$ on, 2 per cent., at beginning of this period | 29 | 66·44 | 1927 | |
| 0·5 | $CHCl_3$ 2 per cent. | 27 | 28·08 | 758 | |
| 0·5 | ,, | 13 | 8·22 | 107 | |
| 0·5 | ,, | 15 | 37·26 | 559 | |
| 0·5 | ,, | 8 | 17·95 | 144 | |
| 0·5 | ,, | 11 | 52·61 | 579 | |
| 0·5 | ,, | 13 | 31·1 | 404 | |
| 0·5 | ,, | 15 | 28·5 | 427 | |
| 0·5 | ,, | 16 | 27·26 | 436 | |
| 0·5 | ,, | 9 | 16·58 | 149 | |
| 0·5 | ,, | 13 | 38·36 | 499 | |
| 0·5 | ,, | 12 | 12·35 | 148 | |
| 0·5 | ,, | 12 | 18·22 | 219 | |
| 0·5 | ,, | 9 | 22·74 | 205 | |
| 1·0 | ,, | 30 | 36·99 | 1110 | |

Table III—*continued.*

| Intervals of time during which respiration was counted, in minutes. | Anæsthetic. | Frequency of respiration. | Average depth of respiration. | Total ventilation, in c.c. | Remarks. |
|---|---|---|---|---|---|
| 1·0 | $CHCl_3$ 2 per cent. | 37 | 26·03 | 863 | |
| 1·0 | ,, | 43 | 26·44 | 1137 | |
| 1·0 | ,, | 35 | 25·07 | 878 | |
| 1·0 | ,, | 30 | 25·21 | 856 | |
| 1·0 | ,, | 22 | 19·45 | 428 | Held breath during half this minute. |
| 1·0 | ,, | 35 | 28·77 | 1007 | Held breath for a short interval during this minute. |
| 1·0 | ,, | 38 | 21·65 | 843 | |
| 1·0 | ,, | | not recorded | | |
| 1·0 | ,, | 25 | 15·3 | 384 | Irregular respiration. |
| 1·0 | ,, | 35 | 13·4 | 469 | Very irregular respiration. |
| 1·0 | ,, | 36 | 9·18 | 691 | |
| 1·0 | ,, | 37 | 14·5 | 537 | |
| 1·0 | ,, | | | | Few irregular gasps. |
| 1·0 | ,, | | | | Died. |

*Experiment* 4.—Showing the effect of 3 per cent. of chloroform following a deep and rapid respiration, but not so deep as in fig. 3. Cessation of respiration at the end of 1½ mins. from commencement of inhalation, and subsequent recovery (fig. 4):—

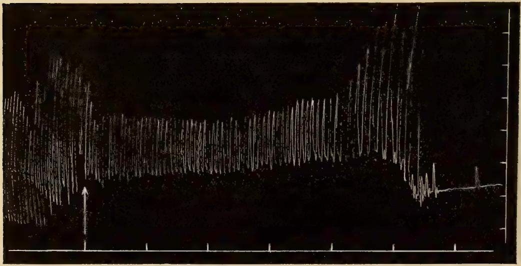


FIG. 4.

Depth of respiration, 1 division = 2·28 c.c.; time intervals, 15 secs. ↑, 3 per cent. chloroform on.

Table IV.—Measurements of Tracing in Fig. 4.

| Intervals of time during which respiration was counted. | Anæsthetic. | Frequency. | Average depth of respiration. | Total ventilation, in c.c. | Remarks. |
|---|---|---|---|---|---|
| 0·25 min. before $CHCl_3$ | Recovery from $N_2O$ | 19 | 77·46 | 1472 | |
| 1st half-min. | 3 per cent. $CHCl_3$ on | 32 | 37·24 | 1192 | |
| 2nd " | " | 25 | 36·6 | 915 | |
| 3rd " | " | 20 | 56·39 | 1128 | Breathing ceased just before end of 3rd half-minute. |
| 4th " | " | 2 | 18·08 | 36 | $CHCl_3$ off after the 4th half-minute. |

*Experiment* 5.—Effect of 3 per cent. chloroform with rapid but comparatively shallow respiration (fig. 5):—

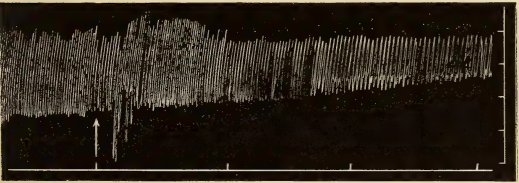


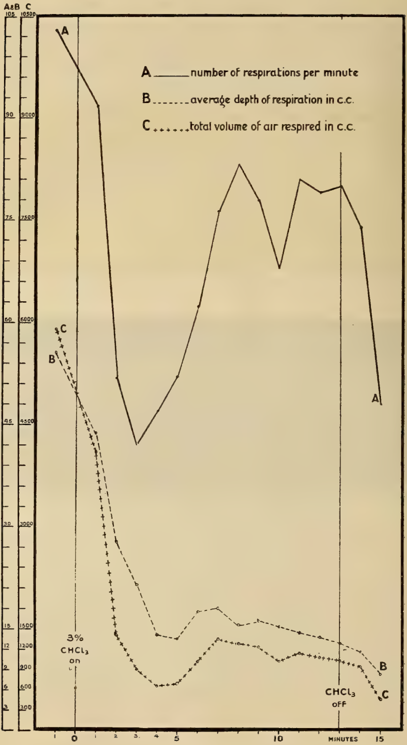
Fig. 5.

Depth of respiration, 1 division = 21·28 c.c. ; time intervals, 30 secs. ; ↑, 3 per cent. chloroform on.

Table V.—Measurements of Tracing in Fig. 5.

| Intervals of time. in minutes. | Anæsthetic. | No. of respirations per minute. | Average depth of respiration. | Lung ventilation, in c.c. |
|---|---|---|---|---|
| 1 | 0 | 106 | 55·38 | 5870 |
| 0·5 | 3 per cent. $CHCl_3$ | 56 | 51·91 | 2907 } 4101 |
| 0·5 | " | 36 | 35·96 | 1294 |
| 0·5 | " | 30 | 31·06 | 932 } 1470 |
| 0·5 | — | — | — | 538 |

The whole data of this experiment are given in Curve II.


A&B
C
105
10500
90
9000
75
7500
60
6000
45
4500
30
3000
15
1500
12
1200
9
900
6
600
3
300
A
A ______ number of respirations per minute
B ....... average depth of respiration in c.c.
C +++++ total volume of air respired in c.c.
B
C
3% CHCl₃ on
CHCl₃ off
A
B
C
1
0
1
2
3
4
5
10
MINUTES
15

Curve II.

*Experiment* 6.—Example in which the respiration is comparatively slow (40 in 30 secs.) and also shallow; average depth, 47·88 c.c., and a low total ventilation of 1915 c.c. per 30 secs. There is no trace of an initial danger-point (fig. 6):—

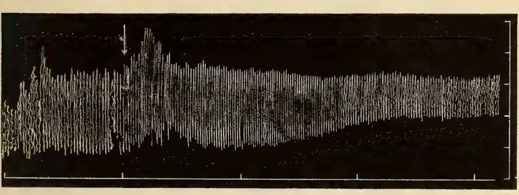


Fig. 6.

Depth of respiration, 1 division = 21·28 c.c.; time intervals, 30 secs.; ↓, chloroform on.

Table VI.—Measurements of Tracing in Fig. 6.

| Intervals of time, in minutes. | Anæsthetic. | No. of respirations per minute. | Amplitude of respiration. | Lung ventilation, in c.c. |
|---|---|---|---|---|
| 0·5 | — | 51 | 54·39 | 2772 |
| 0·5 | — | 52 | 48·82 | 2272 |
| 0·5 | — | 48 | 28·41 | 1364 |

The cat continued to breathe for several minutes without any marked alteration in the type of respiration, and the experiment was then stopped.

It is clear, from the typical experiments quoted, that the initial effect of chloroform is to produce a marked diminution in the average depth of respiration. This occurs during the first few minutes of anæsthesia, but subsequently the depth of respiration becomes constant at a lower level. The rate is not affected to anything like the same extent. In the initial stage a slight increase in the frequency, followed by a decrease, generally occurs, but sometimes a slight decrease takes place at once. The cessation of respiration, which is an initial danger-point in chloroform anæsthesia and may result in death, is the direct effect of deep and rapid respiration prior to the administration of the drug, and the higher the percentage of the drug administered the more likely it is to occur. This can be rendered negligible by a low percentage of chloroform. An examination of many curves has convinced us that some trace of an initial danger-point is rarely absent.

### *Effect on the Lung-ventilation of Re-anæsthetisation of Animals by Chloroform after Recovery from a Previous Anæsthetisation by this Drug when the Reflexes are well marked and Voluntary Movements begin.*

In former papers we have shown that the chloroform content of the blood rises in the initial stage of anæsthesia with great rapidity to a value which approaches a maximum. During this period the amount of chloroform in the blood appears to affect chiefly the respiratory centres, so that the breathing becomes slower, and sometimes ceases altogether, and this is illustrated in the experiments already described. If the animal passes this stage naturally, or recovers on cessation of the anæsthetic, or is revived by means of artificial respiration, then, on continuing the anæsthetic, the amount of chloroform in the blood again rises quickly towards a maximum value, and an equilibrium between the factors which determine the amount of chloroform in the blood is established, the processes of intake and elimination at the pulmonary surface going on side by side.* After the animal passes this first stage of anæsthesia, or if an animal is re-chloroformed after it has so far recovered from a previous anæsthetic that the reflexes have become well marked, it appears to acquire a certain degree of apparent tolerance to the drug, with the result that the drug has a much less effect than would be the case, had not the animal been previously chloroformed. After recovery from chloroform to the point mentioned, the ventilation of the lung takes place at a lower level, both as regards frequency and depth of respiration. If the respiration happens to be deep, the frequency is generally correspondingly diminished. At this lowered level of respiration, the initial effects, when chloroform is again administered, are much less marked, even with very high percentages of chloroform. These points are well illustrated in the following examples :—

*Example I.*—The cat, from which the data of fig. 5 and Curve II were constructed, one minute after cessation of respiration, exhibited asphyxial convulsions, from which it recovered naturally, and, four minutes later, was respiring normally and regularly. Eighteen minutes after cessation of respiration, the animal had so far recovered as to make voluntary movements. It was then re-chloroformed with 4–5 per cent. chloroform. The frequency, amplitude, and total lung ventilation are given in Curve III.

After 10 minutes the animal ceased to breathe for one minute, and the chloroform was taken off, then a series of asphyxial gasps set in for five

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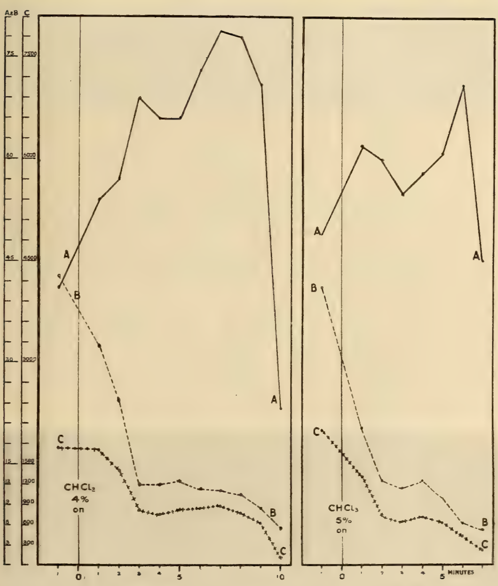
* 'Roy. Soc. Proc.,' 1907, B, vol. 79, pp. 255 and 580.



CURVE III. CURVE IV.

A, B, C as in Curve II.

or six minutes, which passed into normal respiration nine minutes after cessation of respiration.

The respiration data during recovery are given in Table VII.

The animal was then again chloroformed with 5 per cent. chloroform, and the results are given in Curve IV. The animal died in seven minutes.

Table VII.

| | Frequency. | Average depth. | |
|---|---|---|---|
| 9th min. | 20 | 27·66 | 553 |
| The respiration lessened steadily after— | | | |
| 14th min. | 33 | 40·4 | 1333 |
| 17th ,, | 38 | 42·5 | 1615 |
| 18th ,, | 37 | 42·5 | 1572 |
| 20th ,, | Reflexes observed and voluntary movements. | | |

*Example II.*—The animal from which the tracing of fig. 4 was obtained ceased to breathe during the first minute of anæsthesia, and the chloroform was stopped. Asphyxial gasps then set in, which gradually passed into normal respiration. During the 10th minute the respiration was deep, slow, and regular, and at the end of the 12th minute the reflexes re-appeared, and also voluntary movements. The animal was now chloroformed a second time for 8 minutes with 3 per cent. chloroform. There was no evidence of any danger-point. Chloroform was stopped, and the animal allowed to again recover to the same state as above. It was re-chloroformed for a third time with 3·2 per cent. chloroform, and the effect of the drug was still less marked. The first few minutes of the tracings in each case are given below (figs. 7 and 8) and the comparative figures of all three chloroformings in Table VIII.

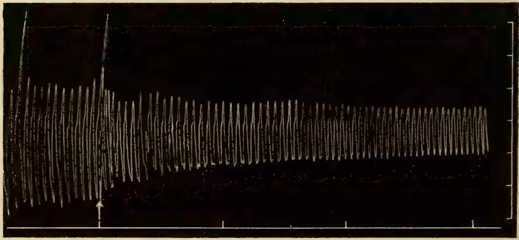


Fig. 7.

Depth of respiration, 1 division = 21·28 c.c.; time intervals, 30 secs.; ↑, 3 per cent. chloroform on.

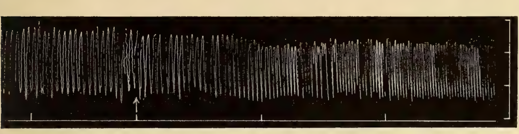


Fig. 8.

Depth of respiration, 1 division = 21·28 c.c.; time intervals, 30 secs.; ↑, 3·2 per cent. chloroform on.

Table VIII.

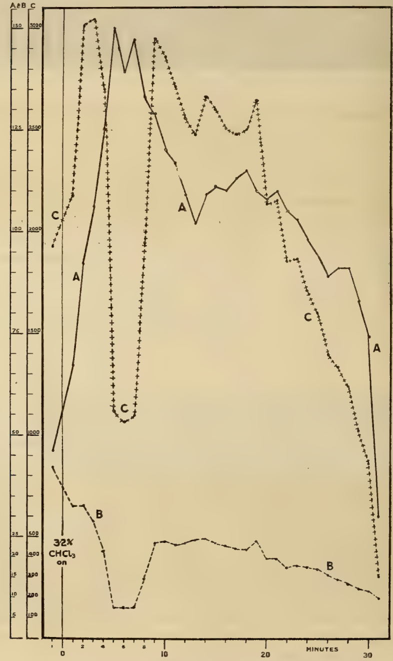
| Intervals of time. | Anæsthetic. | 1st chloroforming. | | | 2nd chloroforming. | | |
|---|---|---|---|---|---|---|---|
| | | Frequency. | Amplitude. | Ventilation. | Frequency. | Amplitude. | Ventilation. |
| 1 min. before CHCl$_3$ | None | 76 | 77·46 | 5887 | 32 | 83·63 | 2676 |
| 0·5 min. | CHCl$_3$ on 3 per cent. | 32 | 37·24 | 1102 } 2017 | CHCl$_3$ 3 per cent.<br>17 | 49·37 | 839 } 1523 |
| 0·5 ,, | ,, | 25 | 36·6 | 915 | 18 | 38·30 | 684 |
| 0·5 ,, | ,, | 20 | 56·39 | 1128 } 1164 | 22 | 32·56 | 716 } 1445 |
| 0·5 ,, | ,, | 2 | 18·08 | 36 | 25 | 29·15 | 729 |
| 3rd ,, | CHCl$_3$ off. | A few gasps | asphyxial. | | 65 | 24·54 | 1660 |
| 4th ,, | ,, | ,, | ,, | | 81 | 23·4 | 1895 |
| 5th ,, | ,, | ,, | ,, | | 89 | 21·28 | 1894 |
| 6th ,, | ,, | ,, | ,, | | 89 | 20·64 | 1837 |
| 7th ,, | ,, | | | | CHCl$_3$ off. | | |

| Intervals of time. | Anæsthetic. | 3rd chloroforming. | | |
|---|---|---|---|---|
| | | Frequency. | Amplitude. | Ventilation. |
| 1 min. before CHCl$_3$ | None | 46 | 41·92 | 1928 |
| 0·5 min. | CHCl$_3$ on 3·2 per cent. | 25 | 38·3 | 958 } 2182 |
| 0·5 ,, | ,, | 42 | 29·15 | 1224 |
| 0·5 ,, | ,, | 92 | 32·77 | 3015 |
| 0·5 ,, | | | | |
| 3rd ,, | ,, | 106 | 28·73 | 3045 |
| 4th ,, | ,, | 125 | 21·57 | 2699 |
| 5th ,, | ,, | 150 | 7·45 | 1117 |
| 6th ,, | ,, | 139 | 7·66 | 1065 |

The complete course of the third chloroforming, which was continued until death in 31 minutes, is shown in Curve V.

No doubt the early cessation of respiration in the first chloroforming was largely due, as we have pointed out, to the excessive lung ventilation prior to the anæsthetic, combined with the high percentage of chloroform;

in subsequent chloroformings the respiratory centres are depressed to such an extent that the respiration is at a lower level, and consequently



Curve V.—A, B, C as in Curve II.

identical or even higher percentages of chloroform have a less effect on the ventilation, and no sign of any initial danger-point is present.

*Example III.*—In the following experiment the animal was anæsthetised under a bell-jar with chloroform, the necessary operative procedures carried out, and then placed in the plethysmograph and allowed to recover until all the reflexes were well marked and voluntary movements returned. When the respiration was steady and equable, 2·5 per cent. of chloroform was given, and, as the tracing (fig. 9) shows, there is no very marked effect on the respiration leading to a danger-point. After the third minute the percentage of chloroform was gradually reduced to 1 per cent., and the lung ventilation remained practically the same. After the 10th minute the chloroform was stopped, the animal allowed to recover until the reflexes returned seven minutes later. A high percentage of chloroform (5 per cent.) was now given, and the animal died in nine minutes. A slowing of the respiration occurred at first, but no cessation. Such a percentage administered to an animal not previously anæsthetised with chloroform would almost

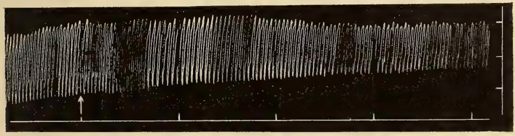


Fig. 9.

Depth of respiration, 1 division = 22·22 c.c.; time intervals, 30 secs.; ↑, 2·5 per cent. of chloroform.

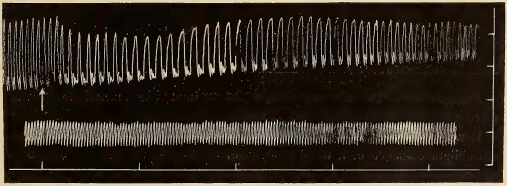


Fig. 10.

Depth of respiration, 1 division = 22·22 c.c.; time intervals, 30 secs. Upper tracing represents the first 2 mins. of 5 per cent. chloroform, the lower the 4th to 6th mins.

certainly have produced death within the first few minutes. This initial slowing was followed by increase in rate and decrease in depth. This is a typical result and well illustrated in the tracing, fig. 10.

The data of this experiment are given in Table IX.

Table IX.

| Intervals of time. | Anæsthetic. | Frequency. | Amplitude, in c.c. | Ventilation. |
|---|---|---|---|---|
| 1 min. before $CHCl_3$ | | 53 | 48·73 | 2705 |
| 0·5 min. | $CHCl_3$ 2·5 per cent. on | 24 | 27·11 | 1131 } 2131 |
| 0·5 " | " | 22 | 45·33 | 1000 |
| 0·5 " | " | 24 | 34·89 | 837 } 1637 |
| 0·5 " | " | 25 | 32·00 | 800 |
| 3rd " | " | 55 | 29·34 | 1614 |
| 4th " | " | 60 | 27·11 | 1628 |
| 5th " | Reduced percentage of $CHCl_3$ | 64 | 24 | 1536 |
| 6th " | 1·3 $CHCl_3$ | 62 | 23·11 | 1433 |
| 7th " | 1·0 " | 54 | 24·39 | 1344 |
| 8th " | 1·0 " | 52 | 29·11 | 1514 |
| 9th " | 1·0 " | 50 | 33·34 | 1666 |
| 10th " | 1·0 " | 49 | 39 | 1905 |
| | $CHCl_3$ off for 8 mins. Reflexes and voluntary movements. | | | |
| 1 " | No $CHCl_3$ | 35 | 46·89 | 1642 |
| 0·5 " | 5 per cent. $CHCl_3$ | 11 | 36·98 | 407 } 705 |
| 0·5 " | " | 9 | 33·11 | 298 |
| 0·5 " | " | 10 | 34·89 | 349 } 744 |
| 0·5 " | " | 14 | 28·22 | 395 |
| 0·5 " | " | 18 | 22·67 | 408 } 891 |
| 0·5 " | " | 25 | 19·33 | 483 |
| 1 " | " | 61 | 18·23 | 1111 |
| 1 " | " | 71 | 16·00 | 1137 |
| 1 " | " | 70 | 15·71 | 1100 |
| 1 " | " | 70 | 15·55 | 1089 |
| 1 " | " | 64 | 14·78 | 946 |
| 1 " | " | 55 | 13·87 | 749 |
| 1 " | " | Ceased to breathe | | |
| 1 " (11th) | " | Asphyxial convulsions | | |

*Example IV.*—In this experiment the animal was anæsthetised with nitrous oxide; the breathing after recovery was regular.

It was then anæsthetised with 2 per cent. of chloroform for three minutes, with slight evidence of a danger-point during the first minute, but the effect was not very marked. The measurements are given in Table X.

Table X.

| Intervals of time. | Frequency. | Amplitude. | Ventilation. |
|---|---|---|---|
| 1 min. before $CHCl_3$ | 55 | 48 ·28 | 2656 |
| 0 ·5 min., 2 per cent. $CHCl_3$ on | 24 | 26 ·86 | 645 } 1161 |
| 0 ·5 ,, | 19 | 29 ·14 | 516 |
| 0 ·5 ,, | 26 | 30 ·71 | 798 } 1755 |
| 0 ·5 ,, | 33 | 29 ·00 | 957 |
| 1 ,, | 64 | 26 ·14 | 1699 |

The animal was allowed to recover for six minutes; when the reflexes had reappeared, 1·5 per cent. chloroform was given for 10 minutes, after which it was increased to 2·5, and finally to 3 per cent. The increasing percentage of chloroform produced an increase in rate and considerable decrease in the average depth, but had a comparatively slight effect on the total ventilation of the lung, as may be seen from the data given in Table XI.

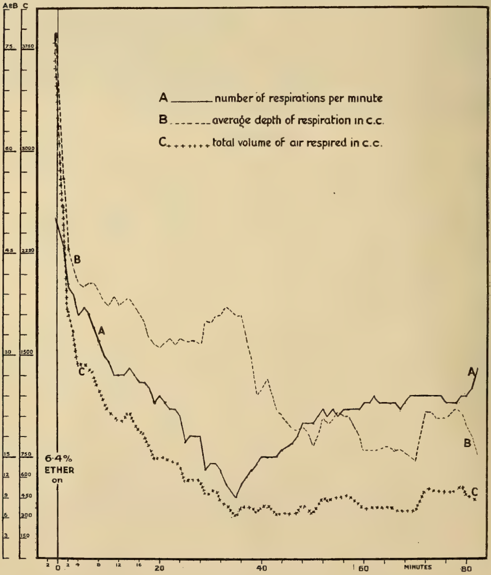
Table XI.

| Intervals of time. | Anæsthetic. | Frequency. | Amplitude. | Ventilation. |
|---|---|---|---|---|
| 0 ·5 min. before $CHCl_3$ | | 30 | 34 ·29 | 1028 |
| 0 ·5 ,, | 1 ·5 per cent. $CHCl_3$ on | 27 | 25 ·71 | 694 } 1225 |
| 0 ·5 ,, | ,, | 24 | 22 ·14 | 531 |
| 1 ,, | ,, | 49 | 21 ·44 | 1050 |
| 1 ,, | ,, | 57 | 22 ·14 | 1129 |
| 4 ,, } Not counted | ,, | | | |
| 5 ,, | ,, | | | |
| 6 ,, | ,, | 65 | 27 ·71 | 1194 |
| 7 ,, } Not counted | ,, | | | |
| 8 ,, | ,, | | | |
| 9 ,, | ,, | | | |
| 10th ,, | ,, | 56 | 18 ·43 | 1032 |
| 11th ,, | 2 ·5 per cent. $CHCl_3$ on | 59 | 16 ·66 | 983 |
| 12th ,, Not counted | ,, | | | |
| 13th ,, | ,, | 56 | 12 ·57 | 830 |
| 14th ,, | 3 per cent. $CHCl_3$ on | 66 | 11 ·57 | 764 |
| 15th ,, | ,, | 60 | 11 ·19 | 738 |
| 16th ,, | ,, | 64 | 10 ·14 | 648 |
| 0 ·5 ,, | ,, | 30 | 6 ·97 | 209 |
| 0 ·5 ,, | ,, | Ceased to breathe | | |

### *Ventilation of the Lung during Narcosis with Ether.*

Adopting the same methods of experiment as with chloroform, a number of plethysmographic records were taken of cats anæsthetised with ether, the percentage of which varied between 6·4 and 14 per cent. These values were controlled by Waller's balance. It was found, on comparing the various tracings, that, as in the case of chloroform, the effect of the drug on the ventilation of the lung depended on the rate and depth of the respiration before the administration of the anæsthetic. The general effect of ether is to reduce the respiration to a lower level. This reduction is at first rapid, and then, after the first few minutes, proceeds slowly. This is illustrated by the two following examples :—

*Example V.*—The cat, weight 3·2 kgrm., was anæsthetised with nitrous oxide, the necessary operations performed, placed in the plethysmograph, and allowed to recover. Ether 6·4 per cent. was administered. The data of the first 10 minutes of this experiment are given in Table XII.



Curve VI.

Table XII.

| Intervals of time, in minutes. | Anæsthetic. | Frequency of respirations. | Amplitude of respiration, in c.c. | Lung ventilation, in c.c. |
|---|---|---|---|---|
| 0·5 | 0 | 25 | 77·14 | 1929 |
| 0·5 | 6·4 per cent. ether | 25 | 59·77 | 1494 } 2584 |
| 0·5 | ,, | 21 | 51·92 | 1090 |
| 1·0 | ,, | 40 | 45·47 | 1820 |
| 1·0 | ,, | 39 | 42·86 | 1672 |
| 1·0 | ,, | 36 | 40·59 | 1461 |
| 1·0 | ,, | 37 | 39·93 | 1478 |
| 1·0 | ,, | 36 | 40·48 | 1457 |
| 1·0 | ,, | 34 | 40·48 | 1358 |
| 1·0 | ,, | 32 | 39·29 | 1057 |
| 1·0 | ,, | 30 | 38·10 | 1043 |
| 1·0 | ,, | 29 | 37·27 | 1081 |

The whole experiment lasted 82 minutes, and, during the last four minutes, a very high percentage of ether was given in order to kill the animal. The whole data of this experiment are given in Curve VI.

*Example VI.*—The procedure was the same as in the last experiment, except that the percentage of ether administered was 13–14 per cent. The measurements are given in Table XIII.

Table XIII.

| Intervals of time, in minutes. | Anæsthetic. | Frequency of respirations. | Amplitude of respiration, in c.c. | Lung ventilation, in c.c. |
|---|---|---|---|---|
| 1·0 | 0 | 75 | 59·96 | 4498 |
| 1·0 | 13—14 per cent. ether | 57 | 30·47 | 1737 |
| 1·0 | ,, | 63 | 26·69 | 1644 |
| 1·0 | ,, | 68 | 25·37 | 1726 |
| 1·0 | ,, | Not counted: even and regular. | | |
| 1·0 | ,, | ,, ,, | | |
| 1·0 | ,, | ,, ,, | | |
| 1·0 | ,, | ,, ,, | | |
| 1·0 | ,, | 46 | 25·05 | 1153 |
| 1·0 | ,, | Not counted: even and regular. | | |
| 1·0 | ,, | 37 | 26·15 | 967 |
| 1·0 | ,, | Not counted: even and regular. | | |
| 1·0 | ,, | 29 | 23·92 | 693 |
| 1·0 | ,, | Not counted: even and regular. | | |
| 1·0 | ,, | ,, ,, | | |
| 1·0 | ,, | 33 | 21·8 | 719 |
| 1·0 | ,, | 31 | 21·59 | 669 |

During the 16th minute the respiration was shallow, and irregular until the 18th minute, when the ether was discontinued.

In no case, with the percentage of ether used, up to 15 per cent., did we notice any cessation of respiration constituting a danger-point, such as is generally found constituting an initial danger-point in chloroform narcosis.

In experiments with re-etherisation after recovery from ether, the effects of the drug on the lung ventilation are less marked, a state of things already referred to in connection with chloroform.

### *General Discussion of Results.*

With unimpeded respiration under anæsthesia by chloroform, given at a slight positive pressure, the ventilation of the lung takes place at a lowered level. Whatever may be the condition of the gas exchange between the alveolar air and the blood, the total exchange of gases between the animal and the atmosphere is diminished in amount, and this continues throughout the whole period of anæsthesia. From our data it will be seen that during chloroform narcosis (in which breathing does not stop) the lung ventilation is diminished in the first three minutes by from 30 to 80 per cent., or on an average about 60 per cent. of its original value, and by a similar amount after prolonged anæsthesia.

It is during this early period that the initial danger-point occurs, and an entire cessation of the respiration may take place, which may result in death.

A simple explanation of this cessation of breathing on administration of chloroform after deep and rapid respiration may, we think, be found in the carbon dioxide content of the blood. The hyperpnœa, prior to the administration of the anæsthetic, probably reduces the carbon dioxide content below normal, so that, as Mosso, Miescher, Haldane and Priestley, and Yandell Henderson* have pointed out, the chemical stimulus necessary to keep the respiratory centre in activity is reduced. The effect of the anæsthetic would be to reduce the excitability of the centre to the effect of the carbon dioxide, so that the quantity of this gas, even after a minute or two of reduced respiration consequent on the administration of the drug, would not be sufficient to maintain respiration, which accordingly would cease. If this is the case, the stoppage of respiration after administration of the drug would obviously depend on two factors, viz., the alveolar ventilation prior to anæsthesia and the strength of the anæsthetic. If the ventilation were sufficient to reduce the carbon dioxide much below normal, and the anæsthetic were strong enough, cessation of breathing would occur; if either of these factors were reduced in a less degree, the effects would vary from slowing of respiration or temporary stopping to a scarcely appreciable change. In all the cases of death at this stage which we have investigated, the heart continued to beat for some little time after respiration ceased.

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* 'Amer. Journ. Physiol.,' 1908, vol. 21, p. 126; 1909, vol. 23, p. 345; 1909, vol. 24, p. 66; 1910, vol. 25, pp. 310 and 385; 1910, vol. 26, p. 260.

In order to throw light on this question, we made analyses of the blood gases of cats under urethane, in which, as the plethysmograph showed, the respiration was extremely regular and constant and the lung ventilation medium in amount. The results are given in Table XIV, and compared with the average of four analyses of the blood of unanæsthetised cats which exhibited marked hyperpnœa, taken from our former paper.*

Table XIV.

| | Composition of the blood gases of cats under urethane in c.c. per 100 c.c. of blood. | | | | Remarks. | Alveolar air. Composition of lung gases. | | |
|---|---|---|---|---|---|---|---|---|
| | Total gas. | $CO_2$. | O. | N. | | $CO_2$. | O. | N. |
| 1 | 60·70 | 49·48 | 10·01 | 1·21 | 10 c.c. of blood | | | |
| 2 | 53·54 | 42·03 | 12·56 | 0·95 | Same cat as 1, 1 hour later | | | |
| 3 | 51·17 | 38·68 | 17·43 | 1·07 | Shallow panting respiration | 5·74 | 9·66 | 84·59 |
| 4 | 60·70 | 49·48 | 10·01 | 1·21 | | | | |
| 5 | 55·54 | 42·03 | 12·56 | 0·95 | Same cat as 4, 1 hour later | | | |
| 6 | 50·01 | 31·77 | 16·68 | 1·55 | | | | |
| 7 | 64·71 | 47·84 | 15·66 | 1·22 | Respiration slow and shallow | 6·24 | 9·15 | 84·69 |
| 8 | 51·57 | 35·76 | 14·89 | 0·91 | | 4·93 | — | — |
| 9 | 51·17 | 38·03 | 12·03 | 1·07 | | 6·25 | 9·74 | 84·02 |
| | Average of four analyses of the blood of unanæsthetised* cats in which the respiration was rapid and deep and the lung-ventilation abnormally great. | | | | | | | |
| | 35·03 | 20·56 | 13·49 | 0·96 | | | | |

* 'Journ. Physiol.,' October 11, 1910, vol. 41, p. 62.

The comparison of these analyses clearly shows that with a deep and rapid respiration the carbon dioxide content of the blood is much less than when the lung-ventilation is normal in character.

Since the dead-space in respiration is constant, it is evident that, with reduced ventilation, a proportionately less amount of inspired air is introduced for diffusion with alveolar air, and consequently the carbon dioxide and nitrogen should accumulate in the alveolar air, and the oxygen content should diminish. These variations, however, could not be very great; at any rate would not vary directly as the change in ventilation. From these considerations we might expect the oxygen content of arterial blood to fall a little below the normal and the carbon dioxide to augment, and to a slight extent also the nitrogen, at about the point of the vanishing of the reflexes in the initial stages of anæsthesia, and in the second stage of anæsthesia. On the other hand, when the animal is recovering from the anæsthetic and the lung ventilation is improving, we might expect the gas-content of the blood at the reappearance of the reflexes to again approach the normal, or even be normal. In Table XV we give analyses, taken from our paper on "The Composition of the Blood Gases in Chloroform Narcosis,"* and the blood gases at the various stages referred to.

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* **'Journ. Physiol.,' Oct. 11, 1910, vol. 41, Nos. 1 and 2, p. 62, Expts. I, II, III, and IV.**

Table XV.—Gas Content of the Arterial Blood of Cats at 0° and 760 in c.c. per 100 c.c. of Blood.

| | | $CO_2$. | O. | N. |
|---|---|---|---|---|
| 1 | Normal (average of six observations) | 25·07 | 13·60 | 1·0 |
| 2 | Disappearance of reflexes (in 3—5 mins.) | 27·76 | 9·52 | 3·05* |
| | | 26·45 | 6·11 | 2·03* |
| | | 34·51 | 7·71 | 1·38 |
| 3 | Re-appearance of reflexes after cessation of $CHCl_3$ for 25—35 mins. | 26·62 | 15·41 | 1·18 |
| | | 26·31 | 12·14 | 1·19 |
| | | 32·83 | 12·58 | 1·28 |
| 4 | Second stage of anæsthesia (average of eight experiments) | 36·00 | 8·14 | 1·49 |
| | Variations ........................ | 16·98—48·38 | 3·51—11·63 | 1·16—2·53 |

* Uncorrected for the nitrogen contained in the oxygen used for combustion of the chloroform.

In normal cats the amount of hæmoglobin, as given by the Gowers-Haldane hæmoglobinometer, lies between 70 and 80, which, in terms of the percentage of oxygen content, would give a value a little above 13·6, the average volume of oxygen found by gas analysis. The fall in oxygen content during the second stage of anæsthesia is about 40 per cent., and in the initial stages often even more than this. The hæmoglobin is then only partially saturated with oxygen during narcosis—indeed not more than 60 per cent. The blood of the normal cat, on the assumption that the alveolar air contains 14 per cent. of oxygen, must, when allowance is made for tension of aqueous vapour at 38° (49·3 mm.) and with 40 mm. carbon dioxide tension in blood, be saturated to the extent of 19 c.c. per 100 c.c. of blood, the oxygen tension being equal to 99·49 mm. But during narcosis the hæmoglobin is in the same state of partial saturation that it would be with a carbon dioxide tension of 40 mm. and an oxygen tension of only 45·5 mm. The diminution of oxygen in the blood during chloroform narcosis seems too great to be accounted for by the lowered level of respiration, particularly in the initial stages, judging by the figures obtained by Loewy and Zuntz and others.*

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* 'Journ. Physiol.,' Nov. 9, 1910, vol. 41, p. 255.

The diminution of oxygen might, however, be to some extent accounted for by a piling up of carbon dioxide in the blood during narcosis, a condition which, as is well known, favours the dissociation of oxyhæmoglobin.

In order to test whether a lowered level of respiration, such as one finds in chloroform narcosis, does result in a marked diminution of oxygen due to the diminished respiration alone, we made the following experiment with a low percentage air-ether mixture, ether being selected as it has a less marked poisonous effect than chloroform :—

A cat was anæsthetised with ether, the necessary operations performed, and placed in the plethysmograph. It was then allowed to recover, and was anæsthetised with 6 per cent. ether-air. Samples of the blood were taken at two stages at which the number of respirations per minute was the same, but the average depth of respiration much less in one than the other.

The following was the breathing during the three minutes before Sample I was taken :—

| | No. of respirations. | Average depth, in c.c. | Total ventilation, in c.c. |
|---|---|---|---|
| 1st min. | 27 | 39·45 | 1065 |
| 2nd „ | 27 | 38·00 | 1026 |
| 3rd „ | 29 | 37·28 | 1084 |

and before Sample II—

| | | | |
|---|---|---|---|
| 1st min. | 27 | 25·22 | 681 |
| 2nd „ | 26 | 25·38 | 657 |
| 3rd „ | 28 | 25·20 | 706 |
| 4th „ | 22 | 26·60 | 585 |

The following results were obtained :—

*Sample I.*—Volume of blood = 10·3 c.c.

Pressure of gas at 13·8° C. and constant volume (48·65 c.c.) = 8·2 cm. ; after addition of oxygen, 23·6 at 13·8° ; after explosion, 23·08 at 13·9° ; after absorption by KOH, 16·14 at 13·9° ; after absorption by "pyro," 0·40 at 13·9° and constant volume (12·60).

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* 'Archiv f. Anat. u. Physiol.,' 1904, p. 166.

*Sample II.*—Volume of blood = 10·3 c.c.

Pressure of gas at 14·2° and constant volume (48·65 c.c.) = 8·98 cm. ; after addition of oxygen, 25·81 at 14·2° ; after combustion, 24·75 at 14·2° ; after treatment with KOH, 16·65 at 14·2° ; and after "pyro," 1·48 at 14·5° and constant volume (12·60 c.c.). The oxygen used for combustion contained 1·3 per cent. of nitrogen.

Composition of Gas in c.c. per 100 c.c. of Blood.

| | Gas. | Ether. | $CO_2$. | O. | N. |
|---|---|---|---|---|---|
| 1 | 48·51 | 1·04 | 36·87 | 9·63 | 0·96 |
| 2 | 53·05 | 2·09 | 39·50 | 10·48 | 0·98 |

Hæmoglobin by hæmoglobinometer: first sample, 70 per cent.; second sample, 68 per cent. This is equivalent to about 12·6 of oxygen.

It is clear from this that a change of total respiration from an average of 1058 to 657 had no effect on the oxygen-content of the blood. It may thus be fairly concluded that the diminution of oxygen-content of blood in chloroform narcosis is not mainly due to diminished respiration, but to the action of the drug on the red corpuscles; since, in agreement with the observations of Pohl and Nicloux, we found that as much as 97 per cent. of the chloroform in blood may be actually associated with the red corpuscles.* The views of B. Moore and Roaf and Gangitano may also be regarded as supporting the idea that there is some direct combination between chloroform and the protein of the corpuscles.

We take this opportunity of thanking the Government Grant Committee of the Royal Society for help in carrying out this work.

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* 'Roy. Soc. Proc.,' 1906, B, vol. 78, p. 450.

# *Factors in the Interpretation of the Inhibitive and Fixation Serum Reactions in Pulmonary Tuberculosis.*

By Alfred H. Caulfeild, M.B.

(Communicated by Prof. T. G. Brodie, F.R.S. Received August 24,—Read November 2, 1911.)

(From the Pathological Department of the National Sanatorium Association, Gravenhurst, Canada.)

Calmette and Massoll[*] have reported that immunisation of a calf with "bacillen-emulsion" has produced, not complement-fixation bodies, but an inhibitive effect which they designate as an "inhibiteur." Previous to this they[†] had reported upon a serum, obtained by intravenous inoculation, which had an extremely active agglutinating property, and under certain conditions was capable of precipitating various solutions of tuberculin. The reaction of fixation was not, however, obtained with either the serum or the serum precipitate. Independently I had observed a somewhat analogous serum reaction (inhibitive reaction) in certain cases of pulmonary tuberculosis, and I first reported this at the meeting of the Canadian Medical Association, Toronto, June, 1910, subsequently publishing more extended investigations[‡] along these lines.

These reactions have become an additional aid in the clinical estimation[§] of the type of case. Emery[||] records an observation which he regards as similar in character to that of Calmette and Massol. The variations in technique at present, however, prevent one from drawing any very close comparisons.

The complement negation phenomenon (inhibitive reaction) becomes more easily demonstrated if one employs a series of antigen dilutions which range, with saline[¶] replacing the serum unit, from one producing a full anti-complementary result to one which may be termed antigenic with the original restrictions of that term. Since my earlier observations on the inhibitive reaction and the reaction of fixation, a number of factors have appeared that have necessitated changes in the technique, in order to obtain more correct interpretations of the end results. While these factors have been mentioned to some extent in the literature upon complement-fixation tests, the importance of their influence has not been brought out.

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[*] Calmette et Massol, "Sur une Nouvelle Réaction Masquant dans les Sérums la Présence d'Anticorps Tuberculeux," 'Bull. de la Soc. de Biol.,' 5 fév., 1910.

[†] Calmette et Massol, 'Sur la Précipitation des Tuberculines par le Sérum d'Animaux immunisés contre la Tuberculose.'

[‡] Caulfeild, "Investigations on Pulmonary Tuberculosis," 'Journ. Med. Research," vol. 24, No. 1.

[§] Caulfeild, "Correlation of Clinical Progress with the Results of Immunological Studies in Pulmonary Tuberculosis," 'Archives of Internal Medicine,' October, 1911.

[||] Emery, "The Immunity Reaction in Diagnoses, especially of Tuberculosis and Syphilis," 'Lancet,' 1911, vol. 1, p. 485.

[¶] When the word saline is used, physiological saline solution is meant (0·85 NaCl in distilled water).

The technique of the inhibitive reaction has been developed with an alcohol-ether extract of the tubercle bacillus, finally prepared as a 2-per-cent. stock solution. Four dilutions of this stock solution are used, the first of which is fully anti-complementary with saline, a 1/10 to 1/20 further dilution gives the antigenic strength, while the intermediate dilutions of 1/2 and 1/4 dilutions represent more or less anti-complementary strengths. With these four dilutions, three main types of end results with pathologic sera become evident.

1. *Inhibitive Reaction.*—With these sera, according to their activity, partial to complete hæmolysis results with the full anti-complementary strength of antigen. Because of certain observations which will be presented I prefer to term this phenomenon the inhibitive reaction, and to assume, for facility of expression alone, that it is evidence of what may be called inhibitin. In contrast to the conception that sensitisers* (amboceptors) show, in the presence of their specific antigens, complement attraction, inhibitin may be said to exert a complement negation effect.

2. *Fixation Reaction.*—Sera with these effects (sensitisers, amboceptors) must cause deflection of complement with the antigenic as well as with all other dilutions.

3. *Indifferent Reaction.*—The essential features here are complete hæmolysis with the antigenic, and no hæmolysis with the full anti-complementary dilution. The varying results which were obtained with the intermediate dilutions and these sera first demonstrated the importance of two factors (serum suspension and natural sheep-corpuscle amboceptor) which should always be controlled. When these are offset, many irregularities, especially noticeable with varying amounts of serum in the inhibitive reaction and the reaction of fixation, are explicable, while "doubtful reactions" also can to a great extent be eliminated.

From the prevalence of tuberculosis in adults, I have been forced to show the specificity of the inhibitive or fixation reactions by the use of other antigens. With antigens such as alcoholic extracts of normal and pathologic organs, specificity has been maintained. Extracts of other micro-organisms have not as yet been tried. From the relative specificity of a serum, obtained after tubercle immunisation, to different acid-fast cultures (as shown by Gengou, Calmette, and others by different serological reactions), it is likely that comparative results would be obtained with the inhibitive and fixation tests between the serum of the tuberculous and other acid-fast bacteria.

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* The terms "amboceptor" and "sensitiser" have been used with the same meaning. In referring to tuberculous fixation bodies, it has been convenient to use the word "sensitiser," reserving the term "amboceptor" for other than tuberculous fixation bodies.

The routine* for some time, as far as this part of the serological work is concerned, has been to determine the reactions of the serum in two strengths (0·3 c.c. and 0·1 c.c.) to the four dilutions of antigen, and subsequently to attempt to show the cause of the various irregularities which may have appeared. For these purposes it is essential that the same samples of sera be used, and that the succeeding reactions be performed immediately, as marked variations have been shown sometimes to take place rapidly in stored sera. To meet these requirements blood is usually drawn from 10 to 20 patients. As a great number of patients have been followed for a considerable period, it is frequently possible to include a number of similarly reacting sera. From these it is often possible to determine conditions connected with the irregularities. The results of these observations and inferences drawn from them can be considered most conveniently under their various headings.

(1) *General Preparation.*—Despite the utmost care and similarity of procedure, I have found that every now and then any single tube may vary remarkably, even to 100 per cent. error, as can readily be proved by a repetition of the test. Further, the results of an entire series have been thought doubtful because of the unsatisfactory condition of some of the controls which, with the usual technique, are not generally made. Because of these inherent characteristics of the test it seems advisable to outline the general technique which has been adopted.

*Glassware.*—These articles are finally cleaned in saline solution, then rinsed in distilled water and allowed to drain and dry. Test tubes, as similar in size as obtainable, are not dried on racks, but packed in wire baskets and inverted on clean filter paper. All articles are sterilised for use.

(2) *System.*—Guinea-pig complement and anti-sheep-corpuscle rabbit hæmolysin have been used. It has been the custom to re-standardise

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* For economy of materials, 0·5 c.c. units have been employed. With this unit it has been convenient to designate in Arabic numerals the number of 0·05 c.c. quantities employed, which are in each instance completed to 0·5 c.c. with physiological saline solution. By this means the insertion of a decimal point converts the protocol into comparison with 1 c.c. units. This is the method that has been adopted in this report, so that actually but one-half the quantities reported have been used.

periodically the hæmolysin to pooled complement of two or more guinea-pigs. Then twice the strength (half the dilution) producing complete hæmolysis in this standardisation is used in the tests. With this amount of hæmolysin, which it is convenient to designate as two units, complement is titrated before each protocol. From these results twice the strength (half the dilution) of complement is used. The sheep-corpuscle suspension of 1/20 is finally prepared by washing three times in 80 c.c. centrifuge tubes. This amount of washing has seemed sufficient to prevent any interference by remaining traces of sheep serum.

(3) *Antigen.*—The inhibitive reaction has, so far, been tried only with an alcohol-ether extract of tubercle bacillus freshly cullured on glycerine broth. It has been prepared as follows:—After the tubercle mass obtained by filtering the glycerine broth cultures is thoroughly washed with saline solution and allowed to dry at room temperature, it is transferred to wide-mouthed 2-litre bottles. The tubercle mass is then covered with a mixture of absolute alcohol (Kahlbaum's) and ether sulph. C.P. (Kahlbaum's) in equal parts, the amount of solvent being 10 to 20 times the weight of tubercle mass. The bottles are shaken frequently during the 5 to 10 days allowed for extraction. Loss of the acid-fast character has in some of the preparations been complete, in others this has been but slight. This has seemed to depend chiefly upon the age of the strain and the capacity for rapid growth. The solution thus obtained has been evaporated at varying rates *in vacuo* over sulphuric acid, and also under a stream of $CO_2$. The yields of 100 c.c., no matter how prepared, have been very close, being in the neighbourhood of 0·2 grm. The solid substance thus obtained is re-dissolved in the same solvent as a 2-per-cent. solution. Both the original and stock solution are passed through double layers of hardened filter paper.

Very considerable differences have been found between the different antigens. Those which have in general given the best results have been obtained from long cultured and rapidly growing strains. It has seemed that an antigen, which in its antigenic dilution gives with suitable sera the most satisfactory complement fixation, is also the most suitable for the demonstration of the inhibitive reaction. Differences in the various preparations have been shown to exist between the range of dilutions that are necessary, on the one hand to produce the full anti-complementary effect with saline, and on the other to act efficiently in antigenic dilutions with sera containing sensitisers (amboceptors). Work in sufficient detail has not been done to correlate completely these end results to the variations in preparation or use of the antigen. Other things being equal, the more non-specific adsorption of complement by the antigen dilution, the more marked will be the amount of apparent complement fixation by suitable sera. For the demonstration of both the inhibitive and fixation reactions considerably more than one unit of available complement should be present to show the specific action of the serum. For use the stock solution is freshly diluted with saline. This gives a cloudy emulsion. The opacity of the different preparations has not always borne a proportionate relation to the anti-complementary effects.

Employing as already mentioned two units of both complement and hæmolysin the full anti-complementary effect with saline takes place in the neighbourhood of a 1/10 to 1/20 dilution. With the determination of this point four dilutions* have been used in the following proportions, namely, 1 c.c., 0·5 c.c., 0·25 c.c., and 0·1 – 0·05 c.c. With these strengths, which I will call for convenience respectively the first, second, third, and fourth dilutions, the antigen and complement controls to any protocol should result about as follows :—

Table I.

| | Freshly diluted antigen. | Saline solution. | Complement. | End results in terms of hæmolysis. |
|---|---|---|---|---|
| 1 | 1·0 c.c. | 1·0 c.c. | (2 units) 1·0 c.c. | None. |
| 2 | 0·5 „ | „ | (2 „ ) „ | Some (± ?). |
| 3 | 0·25 „ | „ | (2 „ ) „ | Complete. |
| 4 | 0·1 „ | „ | (2 „ ) „ | „ |
| 5 | 0·1 „ | „ | (1 unit) 0·5 c.c. | Some (± ?). |
| 6 | Saline | „ | (2 units) 1·0 „ | Complete. |
| 7 | „ | „ | (1 unit) 0·5 „ | „ |
| 8 | „ | „ | (½ „ ) 0·25 „ | Some (± ?). |

Complement is standardised before each protocol, and an amount already defined as a two-unit strength employed. The three controls (Table I) for this are added to each protocol to show the amount of fixative available at the time and under the conditions of the protocol. Considerable variation may take place in a short space of time, so that this has been found advantageous for a satisfactory comparison of different protocols. As each addition is brought to a full unit in all protocols, the effects of the saline dilution are closely related. With alteration of the fixative strength of complement, a corresponding variation will occur in the end results of antigen saline controls. Thus with increase more or less hæmolysis appears in the first tube; with decrease lessened hæmolysis in the second and possibly the third tube. The fifth tube shows as accurately as possible the amount of complement non-specifically affected by the antigenic dilution. Qualitatively the most efficient extract in the antigenic titre is the one which causes the greatest amount of fixation with sera containing sensitisers and, at the same time allows the greatest amount of hæmolysis in the fifth tube (Table I). With the end results of Table I, more than one and less than two units of complement are available for the specific action of inhibitive and fixative sera with antigen.

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* Sachs and Rondoni-Florenz ('Zeits. f. Immunitäts-forschung,' 1909, 1, Abt. 132) call attention to the fact that fractional dilution "often results in a very increased intensity of the reaction." This has in my hands produced marked changes in the antigen controls, and consequently, one may assume, may play a considerable *rôle* in the antigen serum tubes. Particularly with the stronger dilutions fractionally made, one can observe that the upper part carries the greater part of the antigen.

(4) *Nomenclature Adopted in Reading the End Results.*—The need of a comparatively accurate method of recording the end results has been felt by all who have worked with fixation tests, and consequently a number of methods have been devised by Madsen, Epstein, and others. Cloewes photographs the end results; this seems to be the most accurate, although the most time-consuming method. The range of antigen and complement control as outlined in Table I gives one a standard for each protocol, which has seemed to aid in the use of the five expressions in terms of hæmolysis:—1. None (absolutely no hæmolysis). 2. Almost none. 3. Some. 4. Almost complete. 5. Complete (absolutely complete).

A further grading is allowed by the use o the signs + and −, which, however, are probably of value only for the immediate comparison of a set of tubes in the same protocol.

(5) *Effect of Age upon Antigen.*—By thus controlling the antigen for each protocol it has become evident that with time the anti-complementary power increases in an irregular fashion. Thus Extract IV, prepared for use on November 1, began to show towards the end of December tendencies towards incomplete hæmolysis in the third tube (0·25 c.c.). By the end of January this alteration had progressed so far that with two units of complement no hæmolysis resulted in the first three tubes of antigen control. This change has occurred with most preparations, so that the full efficiency of an antigen lasts in the neighbourhood of two months. With this irregular onset of increased anti-complementary properties there is decreased efficiency for the exhibition of the inhibitive reaction. This change applies in a comparable fashion as regards true fixation, if the amount of non-specific fixation by antigen is taken into account. In other words, complete specific fixation with two units of complement may not be produced with suitable sera, if the antigenic dilution be increased and brought to compare with the amount of non-specific fixation produced by the original antigenic dilution. It would

seem that some qualitative change, displaying itself by increased anti-complementary effect, took place, which is not associated with those quantities in antigen which are concerned with specific antigen-serum attraction or negation.

The following protocols (Table II) seem to support this conception, although they are not conclusive. Indeed, it is difficult to realise all the conditions necessary to prove this, nor is it perhaps essential. The sediment (protocol, Table II) was prepared by precipitating the stock solution at 0° C. and re-dissolving this material in an amount of alcohol-ether equal to the amount of stock employed. The filtrate is the stock solution minus the precipitation in the cold. The controls to complement showed that barely two units of fixative were available. Unfortunately it was at this time impossible to make observations with sensitiser sera. Sera 312 and 1354 had the day before given inhibitive reactions with another antigen.

Table II.—19.12.10.

| | | Hæmolysis. |
|---|---|---|
| A.E. IV. (1—10). | | |
| 1·0 c.c. | | None. |
| 0·5 " | | " |
| 0·25 " | | " |
| 1·0 " | S. 312—0·3 c.c. | Almost complete +. |
| 0·5 " | " " | Complete. |
| A.E. IV. Sediment (1—10). | | |
| 1·0 c.c. | | None. |
| 0·7 " | | " |
| 0·5 " | | Almost none +. |
| 0·25 " | | " " |
| 1·0 " | S. 312—0·3 c.c. | Some. |
| 0·5 " | " " | " +. |
| 1·0 " | S. 1354—0·2 c.c. | " |
| 0·5 " | " " | Complete. |
| A.E. IV. Filtrate (1—10). | | |
| 1·0 c.c. | | None. |
| 0·7 " | | " |
| 0·5 " | | Almost none. |
| 0·25 " | | " " +. |
| 1·0 " | S. 312—0·3 c.c. | Some. |
| 0·5 " | " " | " |
| 1·0 " | S. 1354—0·2 c.c. | Complete. |
| 0·5 " | " " | Almost complete. |

(6) *Inhibitive Reaction and its Relation to Time.*—The procedure has been to incubate the first three quantities for one hour and to give the customary

Table III.—Protocol 27.11.10. Extract A.E. IV. Tubes 94 +.

| | | | | Hæmolysis at………………… | 50 mins. | 1 hr. 25 mins. | 2 hrs. 10 mins. | |
|---|---|---|---|---|---|---|---|---|
| A. | Ext. 1·0 c.c. | Salt 1·0 c.c. | Complement 1·0 c.c. | | None | None | Almost none −. | |
| | ,, 0·66 ,, | ,, | ,, ,, | | ,, | Almost complete | Some +. | |
| | ,, 0·5 ,, | ,, | ,, ,, | | ,, | ,, | Almost complete. | |
| | ,, 1·0 ,, | S. 2225, 0·3 c.c. | ,, ,, | | Almost none | ,, − | ,, +. | |
| | ,, 0·66 ,, | ,, | ,, ,, | | Some + | Complete | Complete. | |
| | ,, 0·5 ,, | ,, | ,, ,, | | Almost complete | ,, | ,, | |
| | | | | Hæmolysis at………………… | 20 mins. | 35 mins. | 1 hour. | 1 hr. 25 mins. |
| B. | Ext. 1·0 c.c. | S. 2225, 0·3 c.c. | Cold about 0° C. for 2 hours | Complement added with systemic couplet. | None | None | Some − | Almost complete. |
| | ,, 0·66 ,, | ,, | | | ,, | ,, | Almost complete | Complete. |
| | ,, 0·5 ,, | ,, | | | ,, | ,, | ,, | ,, |
| | ,, 1·0 ,, | ,, | Complement…… | About 0° C. for 2 hours | ,, | ,, | Some + | ,, |
| | ,, 0·66 ,, | ,, | ,, …… | | ,, | ,, | Almost complete | ,, |
| | ,, 0·5 ,, | ,, | ,, …… | | ,, | ,, | Some | ,, |
| | ,, Salt. | ,, | ,, …… | | Almost none | Some | Almost complete | ,, |
| A. | Corpuscles. | Hæmolysin. | Complement. | | Some | Almost complete | Complete | |
| B. | Ext. 1·0 c.c. | Salt 1·0 c.c. | 2 hours at room temp., 30° C. | Complement added with systemic couplet. | None | None | Almost none | Almost none +. |
| | ,, 0·66 ,, | ,, | | | ,, | ,, | ,, | ,, complete. |
| | ,, 0·5 ,, | ,, | | | ,, | Some | Some + | Complete. |
| | ,, 1·0 ,, | S. 2225, 0·3 c.c. | | | ,, | Almost none | ,, | ,, |
| | ,, 0·66 ,, | ,, | | | ,, | ,, | ,, | ,, |
| | ,, 0·5 ,, | ,, | | | ,, | ,, | ,, | ,, |

Serum 2225 was drawn on 25.11.10. Its inhibitive activity tested on 26.11.10.

The sets termed A were put up in triplicate so that the readings were made the following morning at the same time. The sets labelled B were put up in duplicate with a few extra ones with the weaker strengths of antigen. Consequently two readings were made during incubation, and these were aided again by the extra tubes the following morning.

From the control tubes for complement (not given) there appeared to be slightly more than 2 units of fixative strength.

two hours after the addition of corpuscles* and hæmolysin. With this technique I frequently noticed various rates of hæmolysis taking place. The protocol of November 27, 1910, with inhibitive serum 2225 (Table III) shows the results that were obtained in this regard. However, this has not proved a practical method of observing the comparative activity of inhibitive sera, partially because of the importance of the amount of complement available in regard to time effects, and the great difficulty of maintaining in complement a constant two-unit strength.

In Table III are also given the results obtained by leaving antigen-inhibitive serum mixtures in room temperature and in the cold. With these tubes the addition of complement to the mixtures before or at the time of the use of the indicators apparently makes no practical difference.

These results lead to an attempt to dry rapidly antigen-inhibitive serum mixtures, and then determine their effect upon complement as seen in Table IV. Serum 2223 is from a patient which gave previously the

Table IV.—Protocol 13.12.10.

| | | Hæmolysis. |
|---|---|---|
| S. 2223— | | |
| 3 squares macerated in 1·0 c.c. saline | Complement (2 units) 0·5 c.c. | Almost none. |
| 6 " " " | " " | " |
| 10 " " " | " " | None. |
| S. 30 (dog)— | | |
| 3 squares macerated in 1·0 c.c. saline | Complement (2 units) 0·5 c.c. | None. |
| 6 " " " | " " | " |
| 10 " " " | " " | " |
| Antigen only— | | |
| 3 squares macerated in 1·0 c.c. saline | Complement (2 units) 0·5 c.c. | Complete. |
| 6 " " " | " " | Some. |
| 10 " " " | " " | " |
| S. 2223— | Complement added with systemic couplet | |
| 3 squares macerated in 1·0 c.c. saline | | Almost none. |
| 6 " " " | | None. |
| 10 " " " | | " |
| S. 30 (dog)— | | |
| 3 squares macerated in 1·0 c.c. saline | | None. |
| 6 " " " | | " |
| 10 " " " | | " |
| Antigen only— | | |
| 3 squares macerated in 1·0 c.c. saline | | Complete. |
| 6 " " " | | Almost complete. |
| 10 " " " | | " |

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* Hæmolysin and corpuscles have been added separately, that is, previously sensitised corpuscles have not been employed.

inhibitive reaction; Serum 30, from a dog injected with Vaughn's non-toxic tuberculin, gave complement fixation. Both sera were treated as follows: On November 29, 1910, 1·5 c.c. serum was added to 5 c.c. of a 1/10 solution of stock antigen extract, and the mixture brought to 10 c.c. with saline and incubated for one hour at 37·5° C. A control of antigen diluted with saline was treated in the same fashion. After incubation the mixtures were poured on hardened filter paper, and rapidly dried *in vacuo*. Previous to use the paper was ruled by a platinum point into centimetre squares. The results were not expected, and there further seemed to be a shade of hæmolysis hanging about certain squares that were not completely macerated. The repetition with Serum 324 (Table V) was made in identical fashion, except that the paper was thoroughly macerated before incubation. The 10 c.c. mixture, containing 1·5 c.c. serum, was spread over 40 squares (as in the preceding) as evenly as possible. Squares for the test were taken at random. It would seem that the negative attraction of antigen-inhibitive serum mixtures is destroyed by rapidly drying. This contrasts not only with parts of the work already given in Table III, but with the effect

Table V.—Protocol 16.12.10.

| | | Hæmolysis. |
|---|---|---|
| Paper thoroughly macerated and incubated for 1 hour | 2 units complement (0·5 c.c.), 1 hour at 37·5° C. | |
| S. 324— | | |
| 1 square in 1·0 c.c. saline | 2 units complement (0·5 c.c.), 1 hour at 37·5° C. | Complete. |
| 2 ,, ,, | ,, ,, | ,, |
| 4 ,, ,, | ,, ,, | None. |
| 6 ,, ,, | ,, ,, | ,, |
| Antigen only— | | |
| 1 square in 1·0 c.c. saline | 2 units complement (0·5 c.c.), 1 hour at 37·5° C. | Complete. |
| 2 ,, ,, | ,, ,, | ,, |
| 4 ,, ,, | ,, ,, | ,, |
| 6 ,, ,, | ,, ,, | ,, |
| Untreated filter paper— | | |
| 1 square in 1·0 c.c. saline | 2 units complement (0·5 c.c.), 1 hour at 37·5° C. | Complete. |
| 2 ,, ,, | ,, ,, | ,, |
| 4 ,, ,, | ,, ,, | ,, |
| 6 ,, ,, | ,, ,, | ,, |
| Salt 1·0 c.c. Complement 0·5 c.c. | | Complete. |
| ,, 1·0 c.c. ,, 0·25 c.c. | | ,, |

In Tables 4 and 5, 2 hours' incubation were given after the addition of corpuscles and hæmolysin.

of storage. Loss of fixation strength in sera containing sensitiser has been observed to take place within a few days, while storage of inhibitive sera usually causes a less rapid loss of activity. Four inhibitive sera, sealed and frozen in pairs on November 16, 1910, and November 26, 1910, were tested on December 12, 1910. One serum of each date showed full activity, the other two but slight loss.

(7) *Serum Suspension.*—Bordet and Gay have shown that the inhibiting effect of normal rabbit serum in specific hæmolysis "is not due to any effect upon the sensitisation of corpuscles nor to any neutralising action upon complement, but that the addition does oppose the fixation of complement by the sensitised cells." Further, as has been shown, this retarding or hindrance of the specific reaction is offset by increasing the avidity of the antigen-amboceptor for complement, or by dilution with salt. This effect of inert serum in contrast to salt solution as a medium can appropriately be described as serum suspension, and it seems logical to conclude that serum provides unfavourable physical conditions in contrast to salt solution as a suspending medium. Accepting this explanation of the suspension effect of serum, one may suppose that the presence of any serum may tend under favourable circumstances to the end-result of lessened hæmolysis. Certain results strongly support this conception.

With some inhibitive sera irregularities in the end result with increasing amounts of serum seem explicable on this ground. Thus when 0·1 c.c. serum gives as complete an inhibitive reaction as 0·3 c.c. serum, the end result of the latter can sometimes be increased by further saline dilution. The technique can easily be modified by using more closely related amounts of anti-complementary strengths so that the degree of the inhibitive reaction can be more closely read. In other words, saline dilution encourages specific reaction by off-setting the suspending effect of larger amounts of serum. The anti-complementary effect of antigen can be lessened by saline dilution as well as by increase of the avidity of corpuscles-hæmolysin, so that to some extent the absence of hæmolysis under this condition seems to be due to a suspending effect of antigen upon complement. These effects are partially shown in Table VI, by Serum 1354, which had previously given the same result with 0·2 c.c. as with 0·3 c.c. quantities.

Serum suspension may thus under favourable conditions tend to simulate specific fixation. This makes it essential for one to know in each protocol as accurately as possible how much prevention of hæmolysis serum itself may cause, and, as has been pointed out in the remarks under antigen saline control, how much prevention of hæmolysis by the fourth dilution of antigen before one can feel certain to what extent the combined antigen-serum tube

Table VI.—Protocol 18.11.10.

| | | | | Hæmolysis at end of 2 hours. |
|---|---|---|---|---|
| Ext. IV— | | | | |
| 1·0 c.c. | Saline 1·0 c.c. | | Complement (2 units + ) 1·0 c.c. | Some −. |
| 0·66 " | " | | " " | " +. |
| 0·5 " | " | | " " | Almost complete. |
| 1·0 c.c. | Saline 2·0 c.c. | | Complement (2 units + ) 1·0 c.c. | Some. |
| 0·66 " | " | | " " | Almost complete. |
| 0·5 " | " | | " " | " |
| 1·0 c.c. | Saline 4·0 c.c. | | Complement (2 units + ) 1·0 c.c. | Almost complete −. |
| 0·66 " | " | | " " | Complete. |
| 0·5 " | " | | " " | " |
| 1·0 c.c.* | S. 1354, 0.3 c.c. | | Complement (2 units + ) 1·0 c.c. | Almost complete. |
| 1·0 " | " | + saline 2·0 c.c. | " " | Complete. |
| 1·0 " | " | + " 4·0 " | " " | " |
| 0·5 " * | " | | " " | " |
| 0·5 " | " | + " 2·0 " | " " | " |
| 0·5 " | " | + " 4·0 " | " " | " |
| 1·0 c.c.* | S. 10, 0·3 c.c. | | Complement (2 units + ) 1·0 c.c. | Some +. |
| 1·0 " | " | + saline 2·0 c.c. | " " | Almost complete. |
| 1·0 " | " | + " 4·0 " | " " | Complete. |
| 0·5 " * | " | | " " | " |
| 0·5 " | " | + " 2·0 " | " " | " |
| 0·5 " | " | + " 4·0 " | " " | " |
| 1·0 c.c.* | S. 860, 0·3 c.c. | | Complement (2 units + ) 1·0 c.c. | None. |
| 1·0 " | " | + saline 2·0 c.c. | " " | " |
| 1·0 " | " | + " 4·0 " | " " | " |
| 0·5 " * | " | | " " | Almost none. |
| 0·5 " | " | + " 2·0 " | " " | " |
| 0·5 " | " | + " 4·0 " | " " | " |

* These tubes were completed to a unit: as elsewhere the unit was actually 0·5 c.c., so that in reality but half the quantities here quoted were used. Sera 1354 and 10 were inhibitive, and Serum 860 indifferent.

represents specific fixation. From many observations made with sera from man and animals with saline and one unit of complement, it is evident that every variation from complete to no hæmolysis may result. If one takes into consideration the amount of prevention induced by serum and that by antigen a very considerable check is afforded for otherwise "doubtful fixation reactions." This explanation with certain sera (S. 860, Table VI), however, is not sufficient to explain the end results as shown in the first and second dilutions. For here, were the end reactions not specific, the addition of saline would modify them. This serum has on several occasions given no hæmolysis with the first three antigen dilutions. The third and fourth dilutions could not be made in Table VI because of lack of serum. The increased anti-complementary conditions as shown in the third dilution (usually 1/40 or

1/60) might easily be realised in antigen in a short space of time which, under many methods of control for fixation purposes alone, would escape detection; indeed Serum 860 was in the very earliest part of the work recorded as a positive example of fixation. Since then, because of the repeatedly similar results, and in the light of these conceptions and the clinical correlation, the value of this is felt to be doubtful.

(8) *Natural Sheep Corpuscle Amboceptor.*—This hæmolysin is frequently found in the serum of man and animals, and may interfere with a correct interpretation of the end results. It can be avoided by first treating the serum with corpuscle solution, which, however, means an increase of technique that is particularly objectionable with patients' serum for purposes of clinical correlation. As a routine I have instead used two controls, so that the extent of the influence of this amboceptor under the circumstances of the test may be fairly accurately gauged. These are serum with in one tube the maximum strength of antigen, and in the other saline; saline solution being used to replace the immune hæmolysin with the second incubation. Thus the control with saline gives an end result indicative of the activity of the natural hæmolysin under slightly more favourable conditions than exist in the protocol proper, *i.e.* greater saline dilution. With no hæmolysis in this latter tube it has been shown that slight sensitisation of corpuscles can be produced by adsorption experiments. In only a few instances has the control with antigen shown any hæmolysis. However, no hæmolysis in the antigen control tube for natural amboceptor is not as satisfactory a control as might at first appear, because of the underlying principles, which can be shown by the apparent degree of the inhibitive reaction before, and the real reaction after, adsorption of the amboceptor. This is chiefly due to the fact that the curve of the end results with regard to time and degree is not in direct proportion to increase of the different biological constituents of the test. In this regard, the use made of the term one and two units is inaccurate; such nomenclature is, however, convenient, and is in this way qualified.

In earlier parts of the work a negative result in the antigen control tube was relied upon too extensively, and certain of the interpretations made were probably not correct. With any more than "some" hæmolysis in the saline tube, adsorption of the natural amboceptor should be performed for an accurate estimation of the amount of inhibitin.

The presence of natural amboceptor seems to explain certain irregularities of the inhibitive reaction obtained with varying amounts of serum and anti-complementary antigen. Thus a serum may show no evidence of inhibitin with 0·1 c.c. and a full reaction with 0·3 c.c., while the controls show the presence of the natural hæmolysin. After adsorption, the serum would show lessened or no evidence of inhibitin with 0·3 c.c. Besides this apparent increase, a serum without inhibitin may erroneously be credited with that reaction, as the hæmolysis can be shown to be due to the natural amboceptor, although the control tube with antigen showed no hæmolysis. This agrees with what was earlier stated, namely, that anti-complementary strengths of antigen act partially by suspending complement, and can be offset by increasing the avidity of the corpuscle-amboceptor complex.

Apart from the inhibitive reaction, this factor may hinder true fixation results. This is best illustrated when 0·1 c.c. serum shows no hæmolysis, while 0·3 c.c. gives a trace. Thus in a serum with strong natural amboceptor effects, specific fixation may be partially masked and the results imperfectly interpreted.

Again, in sera that have been classed as indifferent, all degrees of hæmolysis with the second and third dilutions of antigen have been observed. After adsorption, the serum in nearly all instances shows no hæmolysis with the first three dilutions, the explanation being already given as serum suspension in addition to antigen adsorption. In fact, in interpreting the results obtained with sera untreated (beyond inactivation), regard must be paid to the controls for both serum suspension and the natural amboceptor for corpuscles.

From many observations upon the presence and variation of natural amboceptor in the serum of patients in all stages of nutrition, the results have suggested that a study of these bodies* might prove of value in digestion and assimilation experiments.

The technique that has been adopted for adsorption experiments with small amounts of serum has been as follows :—

Corpuscle solution (1/20) 5·0 c.c. + serum 2·0 c.c. are incubated for 1½ hours, centrifuged, and the supernatant fluid removed by a pipette. Proportionately smaller amounts can be used where the amount of serum is less. This serum-saline mixture contains nearly 0·15 c.c. serum in 0·5 c.c., which corresponds to the unit and actual amount used. The serum treated corpuscles are brought to twice the original amount of solution (*i.e.*, 10 c.c.) and activated by 10 units of complement. Table VII illustrates the variation that may obtain with both a partially inhibitive and a fixation positive serum. Table VIII shows the effect of the natural hæmolysin with indifferently reacting sera, and several sera with varying amounts of inhibitin.

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* McGowan ('Journ. Path. and Bact.,' 1911, vol. 15, No. 3), has shown that when rabbits are fed on ox-blood a hæmolysin, an agglutinin, and a precipitin against ox-blood are produced.

Table VII.—27.3.11.

| | Antigen XII (1/10). | Hæmolysis before adsorption. | After adsorption. | | Hæmolysis before adsorption. | After adsorption. | Antigen and salt control. |
|---|---|---|---|---|---|---|---|
| 1 | 1·0 c.c. S. 312, 0·3 c.c. | Almost complete | Some | S. 861, 0·3 c.c. | None | — | None. |
| 2 | 0·5 ,, ,, ,, | Complete | Almost complete | ,, ,, | ,, | — | Almost none. |
| 3 | 0·25 ,, ,, ,, | ,, | Complete | ,, ,, | ,, | — | Complete. |
| 4 | 0·1 ,, ,, ,, | ,, | ,, | ,, ,, | Almost none + | None | Complete (with 1 unit of complement some). |
| 5* | 1·0 ,, ,, ,, | Almost none | — | ,, ,, | None. | — | |
| 6* | Salt ,, ,, | Complete | None | ,, ,, | Some. | — | |
| 7† | ,, ,, ,, | Some + | Some — | ,, ,, | Almost none + | — | |
| 8 | 1·0 c.c. S. 312, 0·1 c.c. | None | — | S. 861, 0·1 c.c. | None. | — | |
| 9 | 0·5 ,, ,, ,, | Almost none | — | ,, ,, | ,, | — | |
| 10 | 0·25 ,, ,, ,, | None | — | ,, ,, | ,, | — | |
| 11 | 0·1 ,, ,, ,, | Complete | — | ,, ,, | ,, | None. | |

* Tubes 5 and 6 received saline instead of immune hæmolysin of system in the second incubation.

† Tube 7 received one-half the quantity of complement, as also did a second control of the 0·1 c.c. antigen and salt tube.

Table VIII.—5.3.11.

From the previous protocol Sera 1372, 860, and Rabbit 106 were classed indifferent, the others gave evidence of varying amounts of inhibitin.

| Antigen. | Sera. | Hæmolysis. | Hæmolysis of corpuscles sensitised by adsorption. |
|---|---|---|---|
| 1·0 c.c.<br>0·5 " | S. 1372, 0·3 c.c.*<br>" " | Almost none<br>Some + | Almost complete. |
| 1·0 "<br>0·5 " | S. 870, 0·3 c.c.<br>" " | Almost none +<br>Almost complete | Some. |
| 1·0 "<br>0·5 " | S. 860, 0·3 c.c.<br>" " | None<br>None | Almost complete. |
| 1·0 "<br>0·5 " | S. (Rab.) 106, 0·3 c c.<br>" " | None<br>Some | Complete. |
| 1·0 "<br>0·5 " | S. 302, 0·3 c.c.<br>" " | Some +<br>Complete | Some. |
| 1·0 "<br>0·5 " | S. 1870, 0·3 c.c.<br>" " | Almost complete<br>Complete | None. |
| 1·0 "<br>0·5 " | Saline, 1 c.c.<br>" | None<br>Almost none + | None. |

* 0·3 c.c. was obtained by using 0·5 c.c. of saline serum mixture containing approximately 0·15 c.c. serum.

(9) *Variation in the Fixative Power of Fresh Guinea-pig Serum.*—Unfortunately, it has not always been recorded in standardising complement whether this was from one or more animals.

Lately, however, some endeavour has been made to note this, and, in 24 instances, when the complement was from only one animal, the following standardisations were made:—

| | | | | | |
|---|---|---|---|---|---|
| 1 unit strength in a | 1/15 dilution | or less | in | 4 | guinea-pigs. |
| 1 | " | 1/20 | " | " 11 | " |
| 1 | " | 1/40 | " | " 6 | " |
| 1 | " | 1/40 | " | over 3 | " |
| | | | Total...... | 24 | " |

This method of estimating the fixative power of complement is not satisfactory, for in the few examples of sera which, in dilutions of 1/20, that did not completely hæmolyse the corpuscles, the 1/10 dilution had a very rapid effect; and in this latter dilution seemed efficient in the succeeding protocol, although, according to the method of standardisation, did not contain two units.

Apparently, reproduction of the fixative strength of serum may take place by the addition of fresh serum.

The following results in this connection have been obtained:—

February 16, 1911.—Fresh serum from two guinea-pigs killed on February 15, 1911, standardised 1/20 dilution to 1 unit; used satisfactorily in a 1/10 dilution in the succeeding protocol.

February 18, 1911.—About 12 c.c. of fresh guinea-pig serum (drawn February 17, 1911) added to between 3 and 4 c.c. of serum remaining from the preceding lot. Immediately after adding the fresh to the older serum the mixture standardised at barely 1/20. Within 3 hours this was used in a 1/10 dilution. The protocol of 18 sera contained well-marked examples of fixative positive sera, and yet nothing less than partial hæmolysis resulted, in a few instances, with the higher strengths of antigen and serum. The following morning there remained sufficient of the mixture of guinea-pig sera to show that the fixative strength was over 1/50.

The chief points that have been dealt with may be brought together as follows:—

(1) The technique is concerned with specific biological factors, but is hampered also by other specific and non-specific conditions. For a correct estimation of the specific effect of the antigen-serum complex upon complement these must be adequately controlled.

(2) These factors may be regarded in the light of their tendency to induce or hinder hæmolysis.

A. Conditions favouring no hæmolysis:—

(*a*) Presence of specific tuberculous sensitisers.
(*b*) Non-specific effect of antigen upon complement.
(*c*) " " serum suspension of complement.

B. Conditions favouring hæmolysis:—

(*a*) Presence of specific inhibitin.
(*b*) " natural sheep corpuscle amboceptor in the tested serum.

(3) Effect of saline dilution.

(4) Variations in the fixative strength of complement.

(5) Experimental error incident to the performance of the technique.

# *Preliminary Report upon the Injection of Rabbits with Protein-free (Tuberculo-) Antigen and Antigen-Serum Mixtures.*

By Alfred H. Caulfeild, M.B.

(Communicated by Prof. T. G. Brodie, F.R.S. Received August 24,—Read November 2, 1911.)

(From the Pathological Department of the National Sanatorium Association, Gravenhurst, Canada.)

The injection of animals and the further study of the resulting serological reactions have mainly been made with proteins, and one has tacitly accepted the view that the various phenomena were concerned chiefly with this class of antigen. Previous work had shown that the injection of dogs with Vaughn's non-toxic tubercle residue, which is rich in proteins, produced fixation bodies (sensitisers, amboceptors) to an alcohol-ether extract of tubercle bacilli prepared as described.* Following this it was shown that the injection of this† protein-free extract produced the same result, *i.e.* specific sensitisers. A determination of other biological reactions is in progress, and will be reported in connection with other parts of the work. At present it can be stated that this serum usually is capable of precipitating certain of the tuberculous proteins.

The value of the experimental production and study of various biological reactions in animals is obvious, but it has become almost essential for the logical advancement of the investigation begun on patients. From the data obtained in this manner many considerations suggest that the elective material for correlation of the various immunological states is often to be obtained with clinical normals under exposure and cases of early and slight involvements. With the latter, initial implantations can probably only be obtained in children. It is further desirable that the tuberculous infection be the predominant factor,‡ and not, as often one is forced to conceive, the apex of other pathologic conditions. Obviously the assemblage of such

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* See preceding paper.

† From the method of preparation a few bacilli may be present in the extract. The extent to which this condition may obtain can be shown to have no practical effect.

‡ In this connection, D. V. Hansemann ('Berl. Klin. Wochenschr.,' 1911, vol. 48, No. 1) states that it is not logical to say that the tubercle bacillus is the cause of pulmonary phthisis, as this applies to non-phthisical tuberculosis, notably miliary tuberculosis and acute caseating bronchitis. As regards true tuberculous phthisis, it can only be said that the tubercle is one of the ætiological factors which is certainly essential for the setting up of the typical caseous process, but which is not capable alone of causing phthisis.

Table I.

| Date of injections and serum reactions. | Rabbit 30. Buck, weight 3000 grm. | Rabbit 31. Doe, weight 1245 grm. | Rabbit 32. Buck, weight 2195 grm. | Rabbit 33. Doe, weight 2145 grm. | Rabbit 34. Buck, weight 2350 grm. | Rabbit 35. Buck, weight 1985 grm. |
|---|---|---|---|---|---|---|
| 1.12.10 | Antigen,* 0·2 c.c. in 2 c.c. saline. | | | | | |
| 5.12.10 | Antigen, 0·35 c.c. in 3·5 c.c. saline. | | | | | |
| 10.12.10 | Antigen, 0·6 c.c. in 4 c.c. saline. | | | | | |
| 13.12.10 | Fixation ?† | — | In. | In. | In. | |
| 14.12.10 | — | 1 c.c. S. 324 + antigen mixture.‡ 15 mins. at 37·5° C. | 1 c.c. S. 324 + antigen mixture. 1 hr. at 37·5° C. | 1 c.c. S. 324 + antigen mixture. 30 mins. at 37·5° C. | Antigen, 0·1 c.c. in 1 c.c. saline. | |
| 17.12.10 | Fixation (0·1 c.c.?) | In. | In. | In. | In. | In. |
| 18.12.10 | — | 1·25 c.c. S. 1354 + antigen mixture. 15 mins. at 37·5° C. | 1·25 c.c. S. 1354 + antigen mixture. 1 hr. at 37·5° C. | 1·25 c.c. S. 1354 + antigen mixture. 30 mins. at 37·5° C. | Antigen, 0·125 c.c. in 1·25 c.c. saline. | |
| 22.12.10 | Fixation (0·1 c.c.?) | Fixation | Fixation | Fixation (0·1 c.c.?) | Fixation | In. |
| 25.12.10 | — | 1·5 c.c. S. 1550 + antigen mixture. 15 mins. at 37·5° C. | 1·5 c.c. S. 1550 + antigen mixture. 1 hr. at 37·5° C. | 1·5 c.c. S. 1550 + antigen mixture. 30 mins. at 67·5° C. | Antigen, 0·15 c.c. in 1·5 c.c. saline. | 1 c.c. S. 1369 + antigen mixture. 15 mins. at 37·5° C. |
| 30.12.10 | Fixation | Fixation | Fixation | Fixation | Fixation | In. |
| 3.1.11 | In. | In. | In. | Fixation | Fixation | Fixation. |
| 5.1.11 | — | — | — | — | — | 1·5 c.c. S. 1369 + antigen mixture. 30 mins. at 37·5° C. |
| 14.1.11 | In. | — | Fixation (?) | Fixation (?) | Fixation | Fixation. |
| 3.2.11 | In. | In. | Fixation (0·1 c.c.?) | Fixation (0·1 c.c.?) | Fixation | Fixation. |
| 4.2.11 | Antigen, 0·15 c.c. in 1·5 c.c. saline. | | | | | |
| 16.2.11 | Fixation | In. | — | — | Fixation | Fixation. |
| 27.2.11 | — | — | — | — | Antigen, 0·125 c.c. in 2 c.c. saline. | |
| 3.3.11 | Fixation (0·1 c.c.?) | In. | In. | In. | Fixation (0·025 c.c. serum positive) | Fixation (?). |

* Antigen has been the alcohol-ether extract stock solution. All injections have been given intravenously except that on 10.12.10.

† Fixation; a question mark alone means not absolute fixation with 0·3 c.c. serum; (0·1 c.c.?) means not absolute fixation with 0·1 c.c. serum.

‡ Mixtures were made in following proportions: S. 324, 1·5 c.c. in 5 c.c. saline; antigen, 0·5 c.c. in 5 c.c. saline, mixed and incubated. S. 324 showed a full inhibitive reaction. The other inhibitive sera 1354, 1550, and 1369 prepared in same fashion. All sera in this and other tables were inactivated before use.

In. = indifferent reaction, the variations of which seem to depend chiefly upon the effect of serum suspension and natural amboceptor for sheep's corpuscles.

material is difficult, and even then the work may be hampered by the difficulty of obtaining sufficient quantities of serum with the desired reactions.

It would seem more probable that certain of the larger and more resistant animals would respond in a more analogous fashion to man than rabbits, and it is hoped that later the work may be continued in this manner. In the meantime, however, it seemed desirable to determine certain conditions with rabbits, the results of which may be summarised as follows:—

(1) The injection of rabbits with a saline dilution of an alcohol-ether extract of tubercle bacilli is capable of producing complement-fixation bodies to that antigen.

(2) The digestion at 37·5° C. of the alcohol-ether extract with inhibitive serum (human) for various intervals up to one hour does not affect the production of the specific sensitisers. Examples of both antigen and antigen-inhibitive serum (human) mixtures are given in Table I.

(3) The digestion at 37·5° C. of the alcohol-ether extract with fixation-positive serum (rabbit) for one hour inhibits the production of specific sensitisers (Table II).

Table II.—Injection of Antigen-Fixation Serum (Rabbit) Mixtures

| Animal No. | 1911. | Amount of injection.* | Date serum reaction taken. |
|---|---|---|---|
| Buck rabbit 101, weight 1955 grm. | Feb. 14 | 1·0 c.c. mixture† (0·2 c.c. S. : 0·5 c.c. Antigen A.E., 1/10)‡ | Feb. 17, indifferent reaction.<br>Mar. 3, ,, ,, (Inhibitive ?) |
| | Mar. 10 | 1·0 c.c. mixture (0·3 c.c. S. : 0·5 c.c. Antigen A.E., 1/10) | Mar. 15, indifferent reaction.<br>Mar. 18, ,, ,, |
| | Mar. 30 | 1·5 c.c. mixture (0·3 c.c. S. : 0·5 c.c. Antigen A.E., 1/10) | Apr. 9, no fixation.§ |
| Buck rabbit 107, weight 1990 grm. | Mar. 9 | 1·0 c.c. mixture (0·3 c.c. S. : 1·0 mgrm. bacillus emulsion) | Mar. 15, indifferent reaction.<br>Mar. 18, ,, ,, (Inhibitive ?) |
| Doe rabbit 108, weight 1665 grm. | Mar. 9 | 1·0 c.c. mixture (0·15 c.c. S. : 0·5 c.c. Antigen A.E., 1/10) | Mar. 15, indifferent reaction.<br>Mar. 18, ,, ,, |
| | Mar. 30 | 1·5 c.c. mixture (0·3 c.c. S. : 0·5 c.c. Antigen A.E., 1/10) | Apr. 9, no fixation. |

* All injections were made intravenously.

† Mixtures were incubated for 1 hour at 37·5° C. The fixation positive sera were obtained from the various rabbits (injected with the alcohol-ether extract) whose samples gave at the time complete fixation with 0·1 c.c. at the least.

‡ The proportions are given in terms of 1·0 c.c. amounts. A.E. stands for alcohol-ether extract of tubercle bacilli.

§ Signifies that protocols were formed to test for complement fixation only.

(4) The results of (3) made it essential to determine the effect of digestion with normal rabbit serum and fixation positive (human) serum. At the time

there were no good samples of such human sera available, so that only the results obtained with normal rabbit serum are given in Table III.

Table III.—Injection of Antigen-Normal Rabbit Serum Mixtures.

| Animal No. | 1911. | Amount of injection. | Date serum reaction taken. |
|---|---|---|---|
| Buck rabbit 110, weight 1340 grm. | Mar. 30 | 1·0 c.c. mixture (0·3 c.c. S. + 0·5 c.c. Antigen A.E., 1/10) | Apr. 9, indifferent reaction. |
| | Apr. 4 | 1·0 c.c. mixture (same proportion as preceding injection) | Apr. 24, no fixation ? |
| | Apr. 16 | 1·5 c.c. mixture (same proportion as preceding injection) | Apr. 25 ,, |
| | Apr. 28 | 2·0 c.c. mixture (same proportion as preceding injection) | May 3, complete fixation.<br>May 4, fixation. |
| Doe rabbit 112, weight 2075 grm. | Apr. 11 | 1·0 c.c. mixture (same proportion as preceding injection) | |
| | Apr. 16 | 1·5 c.c. mixture (same proportion as preceding injection) | Apr. 24, no fixation ?<br>Apr. 25 ,, |
| | Apr. 28 | 2·0 c.c. mixture (same proportion as preceding injection) | May 3, complete fixation with 0·1 c.c. serum.<br>May 4, complete fixation with 0·1 c.c. serum. |

(5) The injection of "bacillen-emulsion" in 2, 1·5, 0·5, and 0·005 mgrm. amounts failed to induce fixation bodies. The injection of 100 mgrm. of partially dried dead tubercle bacilli (after alcohol-ether extraction) produced after the first injection sensitisers, so that 0·1 c.c. serum caused complete fixation (Rabbit 105, weight 1635 grm.) although the injection of 0·5 mgrm. failed to do this (Rabbit 105, weight 1815 grm.).

(6) With the amounts and time interval as shown in the tables there has been no evidence of anaphylaxis.*

(7) The technique† followed has already been outlined. The rabbit's blood was obtained by puncture of the ear vein and withdrawal by a 2 c.c. Record syringe. This has been found to be the most rapid and satisfactory method of obtaining up to 5 c.c. of blood. The needle and syringe are sterilised in hot oil, the needle being then well cleared in cold sterile saline.

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* W. M. Scott ("Anaphylaxis in the Rabbit: the Mechanism of its Symptoms," 'Journ. Path. and Bact.,' 1911, vol. 15, No. 1) points out that anaphylactic symptoms depend for their severity upon the amount of precipitate produced in response to the sensitising dose. It is further noted that "the low limit of dose for producing the sensitive condition probably lies about 1 c.c. of blood," and that "the minute dose which it is essential to use in sensitising guinea-pigs towards foreign serum has no effect in the rabbit." It is difficult to compare these necessary amounts of blood quantitatively with the solid substances contained in the alcohol-ether extract, although, as already mentioned, the production of precipitation with tuberculo-protein has been demonstrated by injection of the tuberculo-protein-free antigen.

† *Loc. cit.*

[*Note added November* 23, 1911.—Since submitting these papers in August several articles by Kurt, Meyer, Muck, and others have appeared upon antigens, other than protein, with their effects both in test-tube and injection experiments. Muck* shows that both tuberculo-fat mixtures and tuberculonastin act importantly in both test-tube and living experiments. In contrast to these results Laut† reports that the injection of different tuberculo-products fails in healthy guinea-pigs, rabbits, and goats to produce specific fixation antibodies; in the horse only were these results obtained.]

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* Brauer's 'Beiträge für Klin. Tuberc.,' vol. 20, part 3.

† 'Zeits. für Immunit. Forsch.,' vol. 9, part 2.

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# *Studies in Heredity. I.—The Effects of Crossing the Sea-urchins* Echinus esculentus *and* Echinocardium cordatum.

By Prof. E. W. MacBride, F.R.S., Imperial College of Science and Technology, South Kensington.

(Received November 7,—Read November 16, 1911.)

The manner in which parental characters are transmitted to the offspring when different species of Echinoderms are crossed has been the subject of much experimental enquiry and quite contradictory conclusions have been arrived at by different investigators. Thus Vernon (13), who carried out a most extensive series of experiments with the species of the genera Arbacia, Echinus, Strongylocentrotus, Sphærechinus, and Echinocardium which are available at Naples, came to the conclusion that the condition of the genital glands of the parents (whether imperfectly ripe, fully ripe, or stale) determines in many cases whether or not a hybrid will be formed, and further that though in the majority of cases the hybrid exhibits purely maternal characters, yet it sometimes exhibits paternal characters also, and that this result is also due to the condition of ripeness of the genital glands of its parents. Herbst (5), who also worked at Naples and who used the genera Echinus, Strongylocentrotus, and Sphærechinus for his experiments, found also that the hybrids in many cases showed the paternal influence, but that the extent to which this influence was exhibited varied with the temperature. Doncaster (1), who likewise worked at Naples, also arrived at the conclusion that the greater or less development of paternal characters in the hybrid was due to the temperature. On the other hand, Loeb (7, 8) and his pupil Hagedoorn (4) came to the conclusion that the hybrid exhibited purely maternal characters, and Fischel (2) arrived at the same conclusion on the whole. This conclusion is the more remarkable because Hagedoorn in his experiments used two species of the same genus. Tennent (12) crossed species of the American genera Toxopneustes and Hipponoe and found that the characters of Hipponoe were dominant in the hybrid whichever way the cross was made, but that if the alkalinity of the sea-water were reduced by the addition of dilute acid the influence of Toxopneustes became increased. Lastly Loeb, Redman King, and Moore in a joint paper published quite recently (9), in which they record the results of experiments with the same two species which Hagedoorn used, arrive at the conclusion that the exhibition of paternal and maternal characters in the hybrid is governed by the principle of Mendelian dominance, since, as they assert, the same characters appear in the hybrid whichever way the cross is made, whether, that is to say, in any particular case the character in question is inherited from the male or from the female parent.

During a study of the whole subject which I recently made with the object of summarising the present state of our knowledge of this question of the inheritance of paternal and maternal characters in the hybrid, I was struck with the necessity of a preliminary thorough investigation of the characters of the normal larvæ of the species used in hybridisation experiments. The amount of general acquaintance with Echinoderm larvæ displayed by several of the investigators who have attacked the subject is, to say the least, somewhat defective. Thus Herbst (5), who studied chiefly the cross between Sphærechinus and Strongylocentrotus, attaches great importance to the extent to which lattice-work appears in the skeleton of the arms of the hybrid. In the normal larva of Strongylocentrotus, it is true, all four arms are supported by unbranched calcareous rods, whilst in the normal larva of Sphærechinus, each of the two posterior arms is supported by parallel rods connected by cross-pieces like the steps of a ladder, an arrangement which is termed "lattice-work." But Herbst fails to take into account the fact that in the normal larva of Strongylocentrotus a lattice-work skeleton can appear as a variation, and hence an attempt such as he makes to estimate quantitatively the influence of one parent by the amount of lattice-work which appears in the hybrid rests upon an insecure foundation.

There are, however, two cases known to me where the larvæ of two species which can be crossed differ from one another in unmistakable features, about the presence or absence of which there can be no possible doubt. These are, (1) the case of the species *Echinus esculentus* and *Echinus miliaris*, (2) the case of the species *Echinus esculentus* and *Echinocardium cordatum.*

The first case has been investigated by Shearer, De Morgan, and Fuchs in a paper just published (11). As I showed in 1899 (10), the larvæ of these two species are distinguishable in the later stages of their development by the number and arrangement of the "ciliated epaulettes." These epaulettes are loops of the longitudinal band of ciliated ectoderm common to all Echinoderm larvæ which acts as their locomotor organ. These loops, when the larvæ are about two weeks old, become cut off from the rest of the band and form four horizontally-placed crescents of ciliated ectoderm arranged in a circle round the body of the larva, and in the later stages of development the main work of locomotion is thrown upon them. In the larva of *Echinus miliaris,* a bright patch of green pigment is formed posterior to each epaulette; of this pigment there is no trace whatever to be found in the larva of *Echinus esculentus.* On the other hand, in the larva of *Echinus esculentus,* when it is about three weeks old, an additional pair of ciliated epaulettes is formed, which are situated nearer the aboral pole of the larva. No trace of these extra epaulettes is to be found in the larva of *Echinus miliaris.* Finally, in the larva of *Echinus esculentus,* when it is about four weeks old, a pedicellaria makes its appearance at the aboral pole; no trace of this pedicellaria is to be found in the larva of *Echinus miliaris,* though in both larvæ lateral pedicellariæ are developed.

Now, Shearer, De Morgan, and Fuchs find that the hybrid larva, with respect to the three characters just enumerated, viz., epaulettes, pigment, and pedicellariæ, is always purely maternal, whether the male parent be *Echinus miliaris* or *Echinus esculentus.* In fact, the larvæ have the character they should have had if the eggs from which they took their origin had been normally fertilised. This result is quite startling, but, as the experiments have been repeated again and again and checked in every possible way, it may be taken as well established.

Case No. 2, *i.e.*, the cross between Echinus and Echinocardium, had already been included within the scope of Vernon's investigation (13). He crossed the eggs of *Echinocardium cordatum* with the sperm of Echinus, Strongylocentrotus, Sphærechinus, and Arbacia, and obtained in each case about half as many larvæ as when the eggs were fertilised with the sperm of the same species. The hybrid larvæ were all of the maternal type, but the aboral spike (which will be described later) was considerably shorter in them than in the normal larvæ. In most cases, attempts to fertilise the eggs of other genera with Echinocardium sperm were entirely unsuccessful, but in one case, when the eggs of Echinus were used, one-third of them developed and produced larvæ of the purely maternal type.

The experiments, the results of which are recorded in this paper, were performed with the species *Echinocardium cordatum* and *Echinus esculentus.* The results which were obtained are almost at total variance with those which Vernon records. A word or two, therefore, on the conditions under which the experiments were carried out may be in place.

In the end of June, 1911, I went to Millport, on the Clyde, and through the courtesy of the Director, Mr. Richard Elmhirst, I was accorded the use of a table in the Biological Station of the West of Scotland Marine Biological Association. I desire to record my gratitude to the Director and also to Dr. Gemmill, Vice-President of the Association, for the help they gave me in my experiments. My thanks are also due to the staff of the Zoological Department of the University of Glasgow, from whom I obtained the loan of apparatus. I remained at Millport during the months of July and August, and had abundance of the urchins of both species at my disposal. In fact, at low spring tides, Echinus and Echinocardium could both be obtained by the bucketful within a comparatively short distance of the laboratory. Both species were sexually ripe, and in both cases I was able to rear the normally fertilised eggs through a great portion of their developmental cycle.

In the case of *Echinus esculentus* the larvæ lived for four weeks and developed their epaulettes, and all the eight larval arms. Doubtless it would have been an easy matter to rear them through their metamorphosis into the adult form, but as I had previously worked out the development of this species in great detail I gave no special heed to the larvæ.

In the case of *Echinocardium cordatum,* however, I was able to rear large numbers of the larvæ through the whole of their larval development, and saw them metamorphose into young urchins under my eyes. I used a culture of the diatom Nitschia as food in the case of both species, and for this I am indebted to Dr. Gemmill.

Now the artificial fertilisation of the eggs of Echinocardium which Vernon carried out resulted in the production of only a comparatively small proportion of larvæ, and these lived at longest only about eight days. It may be added that, according to my experience, all Vernon's larvæ were sickly, and their development went forward very slowly. In my cultures, larvæ three days old were more advanced in development than his larvæ when they were five days old. I think, therefore, that it will be conceded that my material was in a much healthier state than that which was at Vernon's disposal.

In fig. 1, a pure-bred larva of *Echinocardium cordatum* six days old is represented. It will be observed that the rudiments of eight larval arms are already to be seen. The "post-oral," or "anal" arms (as they are often designated by German writers), which are the first to develop, are fairly long, and the "antero-lateral" arms (or "oral" arms, as German writers term them), which are the next to develop, are about as long. It may be incidentally remarked that all writers who, up till now, have dealt with the hybridisation of Echinoderms (with the exception of Shearer, De Morgan, and Fuchs) have ignored all the stages in the development later than the four-armed larva. To judge from much of what has been written on this subject, no one would ever suspect that the larva of an Echinoid had more than four arms; but, as represented in our figure, the six-days-old larva of *Echinocardium cordatum* possesses in addition a pair of "postero-dorsal" arms, and in front of the mouth a pair of small elevations are to be seen which are the first rudiments of the "præ-oral" arms. The œsophagus and the stomach and the cœlomic sacs lying at the sides of the œsophagus are clearly visible. From the aboral pole of the larva a club-shaped appendage projects backwards. This is the distinctive feature of the larva of Echinocardium and its allies. So far as our present knowledge goes it appears to be characteristic of the larvæ of Spatangoidea generally. Turning now to the consideration of the larval skeleton, we note that each post-oral arm is supported by a lattice-work consisting of parallel calcareous rods bound together by numerous cross-bars, but that each antero-lateral arm is supported by a single calcareous rod. The skeletons of both the antero-lateral and the post-oral arm on each side are formed as outgrowths of the "primary calcareous star" which appears on each side of the gastrula in all Echinoida. This star sends back a third process towards the aboral pole of the larva, which is known as the "body-rod." This is situated beneath the stomach. Dorsal to the stomach from the skeleton of the antero-lateral arm there is given off a rod which runs towards the aboral pole parallel to the body-rod, but which does not reach (as yet) so far. This is termed the "recurrent rod." The skeleton of each postero-dorsal arm is also a lattice-work of parallel rods connected by cross-bars, but it originates from a lateral centre of calcification entirely distinct from the primary star. The skeleton of the "aboral spike" owes its origin to the secretory activity of a group of mesenchyme cells wedged in between the ends of the body-rods, which is clearly visible in the larva when it is two days old before the antero-lateral arms have developed, or any external trace of the aboral spike has appeared. The skeleton consists of a lattice work of three slightly diverging calcareous rods bound together by cross-pieces and beset externally by spines. This lattice work is connected with the ends of the body-rods. The aboral spike bears at its apex a cap of columnar epithelium carrying long cilia. The epithelium covering the rest of it is thin, flat, and non-ciliated.

We may compare with this larva the larva of *Echinus esculentus* of the same age. Such a larva is represented in text-fig. 2. We see that the same number of arms are developed as we found in the larva of *Echinocardium cordatum* of the same age. The post-oral arms are, however, longer and the

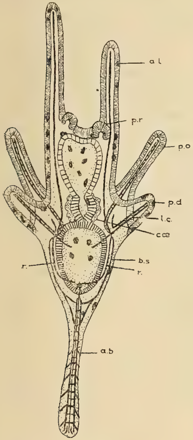

Fig. 1.

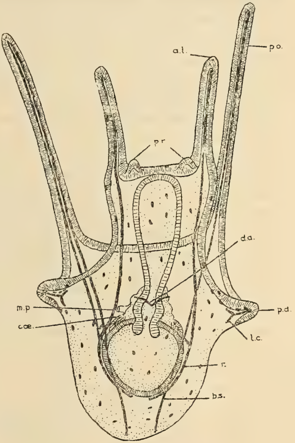

Fig. 2.

Fig. 1.—Larva of *Echinocardium cordatum*, Six Days old, viewed from the Dorsal Aspect.

*a.b.*, aboral spike ; *a.l.*, antero-lateral arm ; *b.s.*, body-rod ; *cœ.*, cœlom ; *l.c.*, lateral centre of calcification ; *p.d.*, postero-dorsal arm ; *p.o.*, post-oral arm ; *p.r.*, præ-oral arm ; *r.*, recurrent rod.

Fig. 2.—A Larva of *Echinus esculentus*, Six Days old, viewed from the Dorsal Surface.

*a.l.*, antero-lateral arm ; *b.s.*, body-rod ; *cœ.*, cœlomic sac ; *d.a.*, dorsal arch ; *l.c.*, lateral centre of calcification ; *m.p.*, madreporic pore ; *p.d.* postero-dorsal arm ; *p.o.*, post-oral arm ; *p.r.*, præ-oral arm ; *r.*, recurrent rod.

postero-dorsal not so far developed as in *Echinocardium cordatum.* The præ-oral arms are indicated merely by slight elevations, but on the dorsal surface of the œsophagus we can see the rudiment of the "dorsal arch," the median centre of calcification, which at a later period of development will provide the skeleton for these two arms. The lateral centre of calcification is not so far developed as in the Echinocardium larva. There is, however, no trace whatever of the aboral spike or of its supporting skeleton, and the post-oral and postero-dorsal arms are supported by single rods and not by lattice-work. In the specimen figured, however, the post-oral arm on the left side is supported by two parallel rods. This is a phenomenon which is by no means infrequent, and Mr. De Morgan has informed me that he has often seen lattice-work in the arms of the larva of *Echinus esculentus.*

The body rods in the specimen figured end in unbranched thickenings, but in many specimens, especially at an earlier period of development, they end in "inbent crooks," which nearly meet at the aboral pole.

We may now examine the result of fertilising the eggs of *Echinocardium cordatum* with the sperm of *Echinus esculentus.* We find that only a small proportion of the eggs so fertilised develop into larvæ. I estimate the number at about 1 in 1,000. These hybrid larvæ can be kept alive for eight days; in no case have I been able to keep them alive for longer than nine days. They grow slowly, and develop only the antero-lateral and post-oral arms. So far from exhibiting exclusively maternal characters they show the influence of the male parent in the most unmistakable manner. One of these hybrids is represented in text-fig. 3, and in it we can observe several paternal characteristics. Thus the aboral spike has totally failed to develop; and this cannot be explained as the effect of a mere retardation of development, because, as was stated above, in the normal larva of *Echinocardium cordatum,* before there is any trace of antero-lateral arms, and when, consequently, the larva possesses only the post-oral arms, and before there is any external trace of the aboral spike, there is to be seen at the aboral pole of the larva a great accumulation of mesenchyme cells, which in the next stage of development give rise to the skeleton of the aboral spike. In the hybrid no trace whatever of such an accumulation of cells at the aboral pole is to be seen at any stage of development. Further, the body-rods of the hybrid show the inbending at the aboral pole which we have seen to be characteristic of the larva of *Echinus esculentus.* The lattice-work of the skeleton supporting the post-oral arms is imperfectly, or not at all, developed; this, too, is a paternal feature. The maternal features which the hybrids exhibit are mainly "size" and "colour." The egg of *Echinocardium cordatum* is less than half the size of the egg of *Echinus esculentus*; it is therefore to be expected that the hybrid which develops from it should approximate in size more closely to the Echinocardium larva than to the larva of Echinus. As a matter of fact, it is considerably smaller than either. The pigment spots of the Echinocardium larva are of a dark red colour, those of the larva of Echinus are of a light red colour. In this respect the hybrid agrees with the maternal parent. It is right to add that, out of all the hundreds of hybrid larvæ reared, I found one solitary case where the aboral spike had been developed; evidently, therefore, the paternal influence is not equally strong in all hybrids, a conclusion which is supported by the very varying extent to which lattice-work is developed in the arms of the hybrid.

Numerous attempts were made to fertilise the eggs of *Echinus esculentus* with the sperm of *Echinocardium cordatum,* in order to obtain the reciprocal hybrid. At first it was thought that success had been attained, for a number of eggs developed into larvæ which showed purely maternal characters. When, however, the precaution was taken of thoroughly sterilising the sea-water in which the fertilisation was made, by heating it to 70° C. and allowing it to cool, none of the eggs developed.

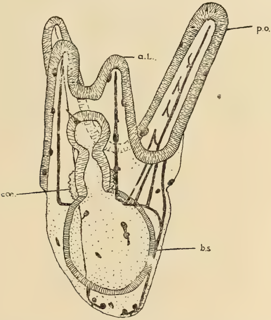

Fig. 3.—A Hybrid derived from an Egg of *Echinocardium cordatum,* fertilised with the Sperm of *Echinus esculentus,* Six Days old, viewed from the Dorsal Surface.
*a.l.*, antero-lateral arm ; *b.s.*, body rod ; *cœ.*, cœlomic sac ; *p.o.*, post-oral arm.

Further, when the eggs of *Echinus esculentus* were allowed to lie in clean sea-water, without the addition of any spermatozoa, a small proportion developed. It is clear therefore that ordinary sea-water, in the summer time, contains enough spermatozoa to cause some of the eggs to develop, a result not to be wondered at when we consider the abundance of *Echinus esculentus,* and the number of males which must be discharging their spermatozoa into the sea-water about the same time. One cannot help wondering whether some of Vernon's results may not have been due to the neglect of the precaution of sterilising the sea-water.

When the eggs of *Echinus esculentus* which have been fertilised with the sperm of *Echinocardium cordatum* in sterilised sea-water are examined under the microscope, many of them are seen to exhibit the fertilisation membrane, showing that the spermatozoa have entered them. Their cytoplasm is seen, however, to be undergoing the form of degeneration known as cytolysis, that is, it is breaking up into globules. One of these eggs is represented in text-fig. 4. Now Loeb (8) has produced the formation of the fertilisation membrane and of the initial stages of development in unfertilised eggs, by treating them with butyric acid; unless, however, they were subsequently treated with hypertonic sea-water, cytolysis set in. He consequently assumes that the spermatozoon has two actions on the egg: it starts cytolysis, to which the formation of the membrane is due, and it also checks the cytolysis which it has started. The spermatozoa of Echinocardium act on the eggs of Echinus like butyric acid without the addition of hypertonic salt solution.

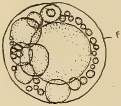

Fig. 4.—An Egg of *Echinus esculentus*, which has been fertilised with the Sperm of *Echinocardium cordatum*.

*f.*, fertilisation membrane.

Loeb (7, 8), Godlewski (3) and Kupelwieser (6) have been able to cause the eggs of sea-urchins to develop, by adding to them the sperm of animals belonging to quite different classes, such as Starfish, Crinoids, and Mollusca, when the alkalinity of the sea-water in which the experiments were made was artificially increased, and in all cases the resulting larvæ exhibited purely maternal characters. In endeavouring to apply this method to the fertilisation of the eggs of Echinus with the sperm of Echinocardium I made mixtures of 100 c.c. of sterilised sea-water with 1 c.c., $1\frac{1}{2}$ c.c., 2 c.c., and $2\frac{1}{2}$ c.c. respectively of an N/10 solution of NaOH in distilled water, and in these mixtures the cross-fertilisation was attempted. In the culture which was made with a mixture of 100 c.c. of sea-water and 2 c.c. of N/10 solution of NaOH a few unhealthy granular blastulæ were found, but none developed into larvæ.

In conclusion, I may point out that the types of sea-urchin represented by Echinus and Echinocardium have been distinct since the beginning of the Secondary epoch and that their common ancestor could not have lived later than a period which a moderate estimate would place at 20,000,000 years ago; yet the germ-cells of the two types will commingle so as to produce a hybrid in which both paternal and maternal characters are represented.

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# *The Physiological Influence of Ozone.*

By Leonard Hill, F.R.S., and Martin Flack.

(Received July 6,—Read December 7, 1911.)

(From the Laboratory of the London Hospital Medical College.)

Ozone has been extolled as the active health-giving agent in mountain and sea air, its virtues have been vaunted as a therapeutic agent, until these have, by mere reiteration, become part and parcel of common belief; and yet exact physiological evidence in favour of its good effects has been hitherto almost entirely wanting. Ozone has been found occasionally in traces in the atmosphere, it has been proved to have active oxidising properties, and on these facts the superstructure of its therapy has been reared.

Popular attention has been fixed on the mysterious and the unknown, and has neglected the prepotent power of cold wind and sunlight to influence the nervous health and metabolism of man. The only thoroughly well-ascertained knowledge concerning the physiological effect of ozone, so far attained, is that it causes irritation and œdema of the lungs, and death if inhaled in relatively strong concentration for any time, *e.g.*, 0·05 per cent., death in two hours (Schwarzenbach); 1 per cent. in one hour (Barlow).

A. Loewy and N. Zuntz[*] write that "the physiological foundations of an ozone-therapy can scarcely be discussed, so little is the extent of our exact knowledge on this subject." The old idea, that ozone passing into the blood acts as an oxidising agent there, thus destroying "organised" and "unorganised" poisons, was exploded by Pflüger,[†] who pointed out that ozone is immediately destroyed on contact with blood; even if it were not, there is no reason why it should oxidise toxins rather than normal constituents of the blood.

C. Binz[‡] observed that "animals submitted to ozone became quiet and appeared to sleep." W. Sigmund[§] also noted this effect in white mice, gold fish, and insects. He considered that ozone is not a very dangerous substance, for even small animals could bear for a time a relatively large amount without serious effect; warm-blooded animals were the more sensitive.

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[*] 'Handbuch der Sauerstofftherapie,' Michaelis, Berlin, 1906, p. 61.

[†] 'Pflüger's Archiv,' vol. 10, p. 251.

[‡] 'Berl. Klin. Wochensch.,' 1882, Nos. 1, 2, 43.

[§] 'Cent. f. Bakter.,' (II), 1905, vol. 14, p. 635.

Filipow* found weak concentrations had no effect on men or animals, while a higher concentration of ozone caused irritation of the respiratory tract.

Schultz† confirmed this irritative effect, and found long-continued breathing of ozone caused pathological changes, particularly in the lungs, which were the cause of death. Schultz considered that the ozone passed into the blood and injured the lung secondarily. Bohr and Maar‡ overthrew this supposition by the ingenious experiment they devised of making one lung breathe ozonised air and the other normal air. They found this lung remained normal while the ozonised lung became œdematous.

Using a concentration of ozone which produced no visible change in the pulmonary structure, these observers found that it caused a diminished uptake of oxygen; the other lung compensated for the deficiency by an increased uptake. This occurred in both cold-blooded (tortoise) and warm-blooded animals. In the former the initial effect of ozone was occasionally a slightly increased oxygen uptake. If the inhalation of ozone were continuous, the increased uptake by the lung ventilated with normal air finally fell away and became deficient; this occurred sooner in the mammal than in the tortoise.

The $CO_2$ output was also diminished, but not so markedly as the oxygen uptake, thus the respiratory quotients often rose over 1. The effect of ozone on the respiratory exchange came on gradually, and with weak concentrations often reached its height after the cessation of the ozone inhalation—there was, in fact, an after-effect which took some little time to pass off. The effect was not modified by a preliminary division of the vagi and pulmonary sympathetic nerves.

The blood of the ozonised animal had no toxic effect when transfused into another. Bohr concluded that the effect was primarily on the lungs, and as the oxygen uptake was affected more than the $CO_2$ output, he claimed that his results supported his view that the pulmonary epithelium by its secretory activity controlled the passage of the respiratory gases. Butte and Peyron§ likewise record that ozone when inhaled diminishes the metabolism.

One of the obstacles in the way of investigation has been the difficulty of obtaining pure ozone free from oxides of nitrogen, and another has been the want of an accurate method of estimating the concentration of ozone. There has been devised lately an ingenious apparatus for producing ozone, which eliminates the production of the oxides of nitrogen, and allows the ready use of ozone for bleaching, sterilising water or ventilating purposes. The ozone is generated by the electrical discharge of high-potential currents across sheets of fine gauze set parallel and insulated from each other. The gauze net ensures the equality of the discharge over the whole surface, and prevents that excessive high-tension discharge at certain rough points, which occurring in the older form of instruments fitted with smooth metal plates, causes the production of oxides of nitrogen from the burning of atmospheric nitrogen.* Our object therefore has been to determine the effects of undoubtedly pure ozone, especially in concentrations far less than those used by previous observers.

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* 'Arch. f. d. Ges. Physiol.,' vol. 34, p. 335.

† 'Arch. f. exper. Path.,' 1882, vol. 29, p. 364.

‡ 'Skand. Arch. f. Physiol.,' 1904, vol. 16, p. 41.

§ 'Comp. Rend. Soc. Biol.,' vol. 46; 'Progrès Médical,' 1894, No. 30, p. 61.

*Method of Estimation of Concentration of Ozone.*—The air containing ozone is sucked by an aspirator or filter pump through a 1 per cent. solution of potassium iodide, acidified with a small quantity of 10 per cent. sulphuric acid contained in a Drechsel wash-bottle. It is essential that contact with rubber be avoided. After 10 litres of air have been passed through the wash-bottle, the acidified KI is removed and freshly prepared pure starch emulsion added. A blue colour indicates the presence of ozone. The amount is estimated by titration with sodium hyposulphite solution until this blue colour is discharged. The hyposulphite solution is prepared by dissolving 22·2 grm. in 1 litre of distilled water, so that 1 c.c. of the solution is equivalent to 100 parts per million of ozone in the air collected as a ten litre sample. For small quantities of ozone the solution may be diluted 10 or 100 times, giving 1 c.c. of the solution equal respectively to ten and one part per million of ozone in the air collected.

*Lethal Dose of Ozone.*—To determine this the animals were placed in a large airtight chamber. The ozonised air was then driven through by means of a gas engine driving an air-pump, and the concentration of ozone determined in the issuing air. The animals could be observed through the glass windows of the chamber, which could also, if necessary, be lighted by electric light. Our experiments show that animals may die after being submitted to 15 to 20 parts per million for two hours. We do not doubt that a lower concentration would have a fatal effect if breathed for a much longer period.

The cause of death is acute inflammation of the respiratory tract. The lungs become intensely congested and œdematous. Microscopically the pulmonary alveoli appear full of an inflammatory exudation. Many of the alveoli are full of blood, for so intense is the irritant effect that hæmorrhages take place. There are no other signs of the effect of ozone in the body. On inhaling ozonised air ourselves and expiring through the iodine test solution we find no evidence of ozone in the exhaled air. It is all taken up by the wet mucous surface of the respiratory tract and exerts its effect there.

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* Mr. Edward L. Joseph, the inventor of the "Ozonair" apparatus, was good enough to give us the use of a complete installation and place his information at our disposal.

Table I.—Lethal Dose. January to March, 1910.

| Animals. | Parts of ozone per million. | Duration of exposure. | Result. |
|---|---|---|---|
| 2 rats | 15 | 3½ hours | *Died* following night, lung showing pneumonia. |
| 2 „ | 2 | 3¾ „ | No ill effects. |
| 2 „ | 3—5 | 3¾ „ | „ |
| 2 rats, 1 cat | 7½ | 3¾ „ | Rats quiet, fur standing up. Recovered. Cat killed next day; signs of lung irritation. |
| 1 dog, 3 rats | 10—20 | 2 „ | Disordered breathing of all animals; all recovered. |
| 2 goats | 9½ | 3½ „ | Jerky breathing; soon recovered. |
| 2 „ | 7 | 3½ „ | Dyspnœic; one had snuffles; soon recovered. |
| 1 dog, 3 rats | 11½ | 2¾ „ | Dog's breath disordered; developed cough and bad breathing 1 hour after. All eventually recovered. |
| 2 goats | 9½ | 3 hrs. 5 mins. | Depressed. Breathing disordered; moist sounds; recovered. |
| 2 rats | 10 | 4 hours | Fur ruffled; recovered. |
| 1 rat | 11 | 2 „ | No permanent ill effects. |
| 1 mouse | 20 | 2 „ | *Died*; pneumonic signs found *post mortem*. |
| 1 „ | 40 | 1 hour | Very disordered breathing; eventually recovered. |

On breathing two to three parts per million, we ourselves find it irritating to the respiratory tract, with a tendency to produce, in this concentration, headache and oppression. The irritation set up by ozone, together with its strong characteristic smell, affords ample warning, and would prevent anyone exposing himself unintentionally to a dangerous concentration. The irritation set up would naturally make anyone remove himself from the influence of the ozone before any serious damage to the lungs had been set up. As far as we can see, then, no serious risk can arise from the use of ozone generators, so long as the generators are not placed in a confined space from which escape is impossible.

It is only possible to estimate concentrations of much less than one part of ozone per million parts of air by passing very large quantities of the ozonised air through the acidified potassium iodide solution. We find concentrations of far less than one in a million parts can be both smelt and tasted; the physiological test for ozone therefore is extraordinarily delicate. If ozone is used in a ventilating system, we think it should be in such concentration as is scarcely perceptible to a keen sense of smell.

Ozone has most potent action as a deodoriser. We tested this by filling our experimental chamber with the smoke of shag tobacco, ammonium sulphide, or carbon bisulphide vapour. At other times we placed in the chamber stinking meat, or human fæces. After putting in action the two small ozonisers, placed in the roof of the chamber, for two minutes, we were not able to detect the odours of these substances. The smell of the ozone masked all other smells. The masking of these smells gives no proof of the destruction of the evil-smelling emanations, for Zwaardemaker has shown that two smells can neutralise each other, *e.g.*, ammonia introduced up one nostril, and acetic acid up the other.

Erlandsen and L. Schwarz* concluded, from a series of careful observations on the effect of ozone on ammonia and hydrogen sulphide, trimethylamine, butyric and valerianic acids, indol and skatol, that the smells are only masked and not destroyed by the presence of ozone. The odoriferous substance and ozone were introduced into the chamber together. After a period the ozone disappeared from the chamber, and the smell was found to have returned. The smell of tobacco, in particular, was masked and not destroyed.

From a hygienic standpoint the ozone may be useful as a deodoriser, since, from the point of view of its effect on the nervous system, it does not matter whether the evil smell is masked or destroyed. The question is which is preferable, the evil smell or the smell of ozone. Certain smells are objectionable, and become more so if persistent and uniform. In cold meat or dry goods stores, tube railways, etc., ozone may have its use as a deodoriser and freshener of the atmosphere, relieving the stale and tedious quality of the air.

In a room fitted with a gas radiator (without flue) we have found, by a series of daily observations, that ozone relieves the disagreeable quality of the air. It seems to give a certain tang to the air, and, by stimulating nerve-endings in the respiratory tract, relieves the monotony of over-warm and close air. We were informed by an engineer employed in a large public office that he added "Sanitas" to the water used for spraying and cooling the air which was pumped into the building on a Plenum system. In the late afternoon the clerks often telephoned down to him and asked for "more Sanitas"—anything to change the monotony of air always warmed to 65° F.

Under the conditions of natural life we are "blown upon by every wind, and wet with every shower." The cutaneous sense organs are submitted to ceaseless flux of physical and chemical conditions, more or less blood and tissue lymph, higher or lower temperature, etc. The heating and ventilating engineer has aimed at giving us in our buildings a uniform summer temperature, unchanged by wind or calm, warm sunshine or cold shadow of the clouds. In the House of Commons the air is drawn in from over the Thames, cooled and wetted by a water spray, and carried in at the rate of 40,000 to 50,000 feet a minute—a fine bracing current. Before it reaches the House it is warmed by passing over steam radiators, mixed, and passed in a uniform draughtless stream at 63° F., through the gauze-covered floor of the House. When the division bell rings the current is switched from the House on to the Division lobbies. Hour after hour the same uniformity is maintained, which leads the open-air man to complain that it is too hot, and the old East Indian to revile the cold. The fault lies in the uniformity. When the House is cleared for division, it should be swept, in our opinion, with a current of cool air straight from the water sprays.

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* 'Zeit. f. Hygiene u. Infections-Krankheiten,' 1910, vol. 67, p. 391.

In such conditions of uniformity an ozoniser, just as a cigarette, may relieve the tedium of the nervous system. Ozonised air may help under the depressing conditions which obtain in many shops and factories by varying the stimulation of the nervous system.

It has been claimed that traces of ozone in the atmosphere, by its oxidising properties, destroy dust, bacteria, noxious gases, and render the air pure. There is no doubt that ozone in the presence of water and in strong concentration is a powerful oxidising agent. It is actually used for the sterilisation of the water supply of certain towns. The ozonised air is thoroughly mixed with the water, and brought into intimate contact with the bacteria. On dry bacteria concentrated ozone has no action (Ohlmüller*). In weak concentrations, such as can be inhaled safely, we found ozone had no sterilising effect when bubbled through moist cultures of *Bacillus coli communis*. The ozone only acts on the surface, and in weak concentrations cannot be expected to pass through relatively thick layers of wet material.

Erlandsen and Schwarz rightly point out that there is no justification for the assertion made by Lübbert that "organic dust, ill-smelling particles and agents of infection cannot exist in the presence of ozone, and that a demonstrable excess of ozone indicates absolute purity of the air."

Owing to its powerful bactericidal action when passed through water in high concentrations, it might be thought that inhalation of ozone would be of value in the treatment of infections of the respiratory tract, and such inhalations have been used, *e.g.*, for pulmonary tuberculosis.

Against the use of all such bactericidal agents in the treatment of pulmonary disease is the fact that the bacilli are growing in the substance of the wet tissues, and therefore to kill the bacilli a concentration must be used which would also kill the tissues.

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* 'Arbeiten a. d. Kais. Gesundheitsamte,' vol. 8, p. 229.

One of the most potent methods of treatment is to draw blood in increased volume to the infected part, by fomentations, blisters, etc., the blood itself having bactericidal and immunising properties. We suggest that inhalation of weak concentrations of ozone, by mildly irritating the respiratory tract, may bring more blood to the part and thus have the curative effect of a fomentation or blister.

Fat has a power of absorbing ozone until it smells strongly of ozone, and it retains the ozone for a long time. Mr. F. Kidd kindly tried for us the application of ozonised lard or vaseline to foul chronic ulcers of the leg, but found that while hot fomentations were efficacious in cleaning up and rendering sweet control cases of ulcer, the ozonised ointment had little effect.

For the investigation of the respiratory metabolism we used the Haldane-Pembrey gravimetric method. Mice or small rats were placed in a beaker fitted with a thermometer and the beaker placed in a Hearson air-bath to keep the external temperature constant. In a first series of experiments the ozone was generated by a specially made small ozoniser, and led partly through the animal chamber and partly through a collecting wash-bottle, in order that its concentration might be determined.

The water vapour given off by the animal varied so much with the passing of urine and fæces, and possibly with the animal putting up its fur, as it does when depressed by the ozone, that we can lay no weight on the calculation of oxygen intake. The weakness of the gravimetric method lies in the fact that the oxygen is calculated from the difference between loss of body-weight and output of water and $CO_2$, and not directly measured.

We shall confine our considerations in these experiments to the loss of body-weight, and the $CO_2$ output. Table II shows the loss of weight sustained by the animal, the amounts of $H_2O$ and $CO_2$ given off before, during, and after ozone. It is seen that there is a marked depression during and after the administration of ozone. The table gives a random selection made from 25 similar experiments.

The figures for the loss of weight represent the loss of weight of the animals weighed in the respiration chamber. Evaporation of urine and fæces when passed contribute to this loss. As the loss of body-weight is also influenced by the passing and evaporation of the urine and water from the fæces, the $CO_2$ output results are the more trustworthy.

As the concentration of ozone given by the small generator seemed to be too high, we obtained the ozone in the following experiments by generating it

Table II.

| | | Air. | Air. | Air. | Ozone. | 1st. After. | 2nd. After. | 3rd. After. | 4th. After. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | Time, in mins. | 15 | 15 | 15 | 105 | 15 | 15 | 15 | 15 | Ozone $1\frac{1}{2}$ parts per million. Breathing noticeably disordered. |
| | Loss of weight | 115 | 121 | 125 | 185 | 55 | 35 | 47 | 46 | |
| | $H_2O$ given off | 125 | 120 | 121 | — | 64 | 45 | 50 | 55 | |
| | $CO_2$ ,, | 87 | 90 | 85 | — | 50 | 33 | 30 | 53 | |
| Mouse | Time, in mins. | 20 | 20 | — | 120 | 20 | 20 | 20 | 20 | Ozone 6 parts per million. Breathing markedly disordered. |
| | Loss of weight | 385 | 258 | — | 499 | 98 | 93 | 83 | 71 | |
| | $H_2O$ given off | 151 | 222 | — | — | 225 | 133 | 94 | 88 | |
| | $CO_2$ ,, | 101 | 86 | — | — | 30 | 21·5 | 22 | 24 | |
| Mouse | Time, in mins. | 20 | 20 | 20 | 90 | 20 | 20 | — | — | Ozone $1\frac{1}{2}$ parts per million. |
| | Loss of weight | 210 | 270 | 210 | 198 | 34 | 150 | — | — | |
| | $H_2O$ given off | 140 | 260 | 200 | — | 76 | 180 | — | — | |
| | $CO_2$ ,, | 121 | 146 | 146 | — | 30 | 50 | — | — | |
| Mouse | Time, in mins. | 75 | — | — | 75 | 75 | — | — | — | Ozone less than 1 part per million. 2 inches from ozoniser. |
| | Loss of weight | 415 | — | — | 335 | 104 | — | — | — | |
| | $H_2O$ given off | 303 | — | — | 344 | 180 | — | — | — | |
| | $CO_2$ ,, | 163 | — | — | 80 | 97 | — | — | — | |
| Mouse | Time, in mins. | 60 | — | — | 60 | 60 | 60 | 60 | — | Ozone well less than 1 part per million. 1 foot from ozoniser. |
| | Loss of weight | 580 | — | — | 515 | 325 | 325 | 380 | — | |
| | $H_2O$ given off | 425 | — | — | 525 | 337 | 335 | 393 | — | |
| | $CO_2$ ,, | 207 | — | — | 64 | 150 | 112 | 208 | — | |
| Mouse | Time, in mins. | 75 | — | — | 75 | 75 | 60 | — | — | Ozone well less than 1 part per million. 100 cm. from ozoniser. |
| | Loss of weight | 405 | — | — | 250 | 185 | 236 | — | — | |
| | $H_2O$ given off | 320 | — | — | 233 | 189 | 135 | — | — | |
| | $CO_2$ ,, | 164 | — | — | 123 | 144 | 115 | — | — | |

in a room, the ozoniser being placed at distances varying from 100 to 350 cm. from the inlet of the ventilation current which was drawn through the animal chamber. Table III gives the loss of weight and amount of $CO_2$ given off in milligrammes in the last eight experiments made under these conditions, the reading for the oxygen as before being variable. In all, 16 similar experiments were made on small rats.

In all these experiments the animals were given a preliminary half-hour or so on air, in which to settle down and adjust themselves to their new surroundings. Judging by the $CO_2$ given off in some cases, the ozone appears to have perhaps a transitory stimulating effect, followed by a corresponding depressant effect; in others there is but little evidence of any action of the ozone at all at concentrations such as these. The ozone itself was always in concentrations far less than one part per million, and varied from day to day according to the atmospheric conditions prevailing. We should state that several of our figures obtained during and after ozone show a R.Q. above 1, confirming the observations of Bohr as to the diminished uptake of oxygen.

Turning to the investigation of the respiratory metabolism of man under

Table III.

| Rat. | | Air. ½ hour. | Air. ½ hour. | Air. ½ hour. | Ozone. ½ hour. | 1st. After ½ hour. | 2nd. After ½ hour. | 3rd. After ½ hour. | 4th. After ½ hour. |
|---|---|---|---|---|---|---|---|---|---|
| A | Loss of weight...... | 102 | 103 | — | 90 | 88 | 74 | — | — |
| | $CO_2$ given off ...... | 132 | 135 | — | 73 | 132 | 129 | — | — |
| B | Loss of weight...... | 119 | 130 | — | 70 | 88 | 144 | 200 | — |
| | $CO_2$ given off ...... | 104 | 120 | — | 130 | 127 | 156 | 108 | — |
| A | Loss of weight...... | 113 | 122 | — | 63 | 57 | 65 | 75 | — |
| | $CO_2$ given off ...... | 91 | 94 | — | 86 | 83 | 100 | 99 | — |
| C | Loss of weight...... | 168 | 155 | — | 190 | 235 | 191 | 122 | 145 |
| | $CO_2$ given off ...... | 173 | 158 | — | 199 | 187 | 182 | 140 | 155 |
| D | Loss of weight...... | 107 | 104 | 103 | 136 | 80 | 97 | 128 | 95 |
| | $CO_2$ given off ...... | 142 | 130 | 129 | 155 | 127 | 130 | 132 | 128 |
| E | Loss of weight...... | 225 | 230 | 215 | 145 | 185 | 123 | 202 | — |
| | $CO_2$ given off ...... | 132 | 135 | 114 | 91 | 97 | 77 | 101 | — |
| F | Loss of weight...... | 145 | 270 | 225 | 235 | 215 | 235 | 165 | — |
| | $CO_2$ given off ...... | 163 | 177 | 162 | 165 | 145 | 137 | 102 | — |
| G | Loss of weight...... | 115 | 195 | 197 | 148 | 177 | 145 | 109 | 149*<br>255 |
| | $CO_2$ given off ...... | 153 | 188 | 200 | 163 | 205 | 173 | 175 | 110*<br>138 |

* Here the upper number represents the result of a further ½ hour of ozone, and the lower numbers that of the succeeding ½ hour in air.

the influence of ozone, we selected the method devised recently by Dr. Gordon Douglas,* of Oxford, owing to its simplicity and efficiency.

The subject was provided with a mouthpiece, fitted with inspiratory and expiratory valves. (We used the excellent mica valves made by Messrs. Siebe, Gorman, and Co., and used in their mine rescue apparatus.) While inspiring atmospheric air, the subject expired into a large canvas-rubber bag of suitable construction, and previously emptied of air. After a period of 10 minutes a fresh bag was substituted, and the volume of expired air in the first bag was measured by pressing the contents of the bag through the meter, and a sample of the expired air was collected and analysed. Successive samples were thus taken, some when the air was ozonised, and some when it was not. The composition of the atmospheric air being known, the requisite data were calculated from the measurements of the meter and the analysis of the samples, all results being reduced to 0° C. and 760 mm.

In all we have made 19 experiments, and append the details of the last seven. In the preliminary trials of the method we found considerable variations in the metabolism in successive periods of time. These were due to want of complete rest on the part of the subject. (See Table IV.)

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* C. G. Douglas, 'Journ. Physiol.,' 1911, vol. 42; 'Proceedings,' p. xvii.

Our last series of experiments were carried out with the subject recumbent on a couch and prepared for the test by a preliminary period of rest. Even then, the opening and shutting of the windows (to vary the condition of ozonisation of the air) must have somewhat influenced our results by altering the cooling effect of the air on the body. These tests were carried out in warm summer weather, and in the final experiment the windows were kept shut all the time and the room ventilated by opening the doors leading into other and larger laboratories. In this experiment we obtained results which we regard as the most conclusive of all.

The metabolism varies with the degree of complete rest of the subject. If he moves slightly more or less, *e.g.*, in reading, talking, this will affect the result, and thus we cannot expect figures more concordant than those we have obtained. Looking at the figures in columns IV and VI, we cannot find any conclusive evidence that ozone altered the respiratory metabolism. Note particularly the final experiment (No. 7), in which the windows were shut and the conditions even all through.

The ozone was given in a concentration that made the air smell quite strongly, and in some cases it was pushed even to an unpleasant degree. Taking these figures together with those obtained on mice, we must conclude that we have failed to obtain certain evidence that inhalation of ozone in weak concentration stimulates the respiratory metabolism, *i.e.*, the output of of $CO_2$ and use of $O_2$. On the other hand, our experiments conclusively show that any considerable concentration of ozone depresses the respiratory metabolism.

We think that the beneficial results obtained by the use of pure ozone in ventilation must be reached by the effect of ozone on the nervous system—by its stimulating the mucous membrane, neutralising smells, and relieving the depressing uniformity of close air. Our experiments show that no harm results to man from breathing air ozonised till the air smells quite strongly of ozone, for periods of half to one hour.

Perhaps the most interesting observation made in the research is this: when the respiratory tract is irritated by ozone, the animal becomes motionless, sits hunched up with its fur erect, thus showing the signs of depression. The ozone lessens the respiratory exchange, reduces it even to one-seventh, at a time when the lung shows no changes visible to the naked eye; the animal adjusts its behaviour to this condition, and keeps very still and quiet. Its body temperature at the same time falls. The damage to the lung cannot be serious, since this depressant effect is quite evanescent.

Table IV.

| I. | II. | III. | IV. | V. | VI. | |
|---|---|---|---|---|---|---|
| Subject breathing for 10 mins. | Amount inspired in litres. | Amount of $CO_2$ in sample. | Amount of $CO_2$ expired per min. | Oxygen in sample. | Corrected amount of oxygen taken in per min. | Remarks. |
| L. W.— | | | | | | |
| Air ...... | 87 ·4 | 4 ·21 | 367 | 16 ·26 | 420 ·4 | Nearly asleep. No effort with tube. Window open. |
| Ozone ... | 95 ·3 | 4 ·31 | 413 | 16 ·09 | 475 ·5 | Reading. Effort of putting on tube. Window shut. |
| Air ...... | 99 ·3 | 4 ·18 | 409 | 16 ·39 | 463 ·6 | Reading. Effort of putting on tube. Window open. |
| L. H.— | | | | | | |
| Air ...... | 107 ·9 | 4 ·21 | 453 | 16 ·63 | 466 ·2 | Windows open. |
| „ ...... | 100 ·3 | 3 ·93 | 393 | 16 ·83 | 419 ·2 | „ |
| Ozone ... | 111 ·6 | 3 ·89 | 431 | 16 ·4 | 515 ·9 | Windows shut. |
| „ ... | 96 ·9 | 3 ·90 | 387 | 16 ·87 | 399 ·2 | „ |
| Air ...... | 131 ·5 | 3 ·96 | 517 | 17 ·07 | 501 | Window open. Bothered by valves. |
| L. H.— | | | | | | |
| Air ...... | 107 ·8 | 4 ·05 | 437 ·6 | 16 ·85 | 441 | Windows open. |
| „ ...... | 100 ·7 | 4 ·00 | 403 | 16 ·75 | 425 ·8 | „ |
| Ozone ... | 86 ·02 | 4 ·05 | 348 | 16 ·39 | 402 ·5 | Windows shut. |
| S. E.— | | | | | | |
| Air ...... | 66 ·43 | 4 ·25 | 282 ·4 | 16 ·195 | 323 ·6 | Reading. Windows open. |
| „ ...... | 58 ·45 | 3 ·85 | 226 ·7 | 16 ·83 | 241 ·9 | „ „ |
| Ozone ... | 58 ·51 | 4 ·34 | 254 ·3 | 16 ·104 | 291 ·1 | „ Windows shut. |
| „ ... | 66 ·95 | 3 ·53 | 236 ·3 | 17 ·49 | 229 ·1 | „ „ |
| M. F.— | | | | | | |
| Air ...... | 83 ·56 | 4 ·15 | 346 ·7 | 16 ·5 | 375 ·2 | Reading. Windows open. |
| „ ...... | 93 ·37 | 3 ·65 | 340 ·8 | 16 ·93 | 382 ·8 | Troubled with mouthpiece slightly. R.Q. 89. |
| Ozone ... | 76 ·95 | 3 ·91 | 300 ·9 | 16 ·36 | 365 ·6 | Reading. Window shut. R.Q. 82. |
| „ ... | 97 ·81 | 3 ·48 | 340 ·3 | 18 ·0 | 274 ·8 | Reading. Troubled with mouthpiece slightly. R.Q. 1 ·25. |
| Air ...... | 70 ·13 | 3 ·99 | 279 ·9 | 16 ·73 | 298 ·8 | Window open. R.Q. 93. |
| A. W.— | | | | | | |
| Air ...... | 68 ·6 | 4 ·19 | 287 ·4 | 17 ·03 | 261 ·3 | Windows open. |
| „ ...... | 74 ·8 | 3 ·48 | 260 ·3 | 17 ·438 | 262 ·3 | „ |
| Ozone ... | 92 ·4 | 3 ·80 | 351 ·2 | 17 ·20 | 341 ·9 | Windows shut. |
| „ ... | 73 ·7 | 3 ·94 | 290 ·4 | 16 ·43 | 341 ·3 | „ |
| „ ... | 67 ·5 | 3 ·80 | 256 ·5 | 16 ·57 | 304 ·5 | „ |
| Air ...... | 60 ·0 | 4 ·15 | 249 ·0 | 16 ·32 | 285 ·0 | Windows open. |
| „ ...... | 45 ·5 | 3 ·91 | 177 ·5 | 18 ·11 | 114 ·9 | „ |
| A. W.— | | | | | | |
| Air ...... | 65 ·9 | 4 ·25 | 280 ·07 | 16 ·15 | 325 | Windows shut all the time. |
| „ ...... | 50 ·2 | 5 ·08 | 265 ·01 | 16 ·19 | 233 ·4 | „ „ |
| Ozone ... | 63 ·2 | 4 ·44 | 280 ·60 | 15 ·91 | 327 ·3 | „ „ |
| „ ... | 56 ·3 | 4 ·236 | 238 ·48 | 15 ·91 | 293 ·9 | „ „ |
| „ ... | 65 ·9 | 4 ·31 | 284 ·02 | 16 ·05 | 331 ·5 | „ „ |
| Air ...... | 63 ·7 | 4 ·31 | 274 ·55 | 16 ·01 | 323 ·6 | „ „ |
| „ ...... | 61 ·8 | 4 ·50 | 278 ·10 | 15 ·93 | 318 ·3 | „ „ |

In pneumonia we see the same thing; the patient is forced by the feeling of illness to keep quiet in bed. How this adjustment is brought about is a subject for further research. It will be of especial interest to see the effect of ozone on the oxygen partial pressure of the blood. We would draw attention to the fact that high pressures of oxygen produce inflammation of the lung (Lorrain Smith, L. Hill, and J. J. R. Macleod) similar to that produced by ozone. It is this resemblance which in part led us to make this research.

### *Conclusions.*

(1) Ozone is a powerful deodoriser. It masks rather than destroys smells. Its practical value in relieving the nervous system from the depressant influence of an unpleasant odour is none the less for this.

(2) A concentration as little as one per million is irritating to the respiratory tract. Exposure for two hours to a concentration of 15 to 20 per million is not without risk to life. The irritative effect and the discomfort produced thereby—cough, headache—give ample warning, and there is no risk from inhaling ozone so long as an outlet for the instinctive escape from its influence is open. It is necessary that systems of ventilation in which ozone is used should be dealt with by those experienced in the matter, so that concentrations may be supplied which will not irritate the respiratory tract.

(3) The respiratory metabolism is reduced by ozone, in concentrations even less than one part per million. There is no conclusive evidence of a preliminary stimulation of metabolism preceding the fall.

(4) The beneficial effect of ozone obtained by the ozone ventilating systems is to be explained by its effect on the nervous system. By exciting the olfactory nerves and those of the respiratory tract and skin, it may relieve the monotony of close air, the smell of tube railways, in cold meat stores, hide stores, and other trades.

(5) There is no harm in breathing weak concentrations of ozone, such as can be scarcely sensed by a keen sense of small.

(6) Ozone in somewhat higher concentrations (one per million) may have some value as a therapeutic agent if inhaled for brief periods; by irritating the respiratory tract it may act as a blister or fomentation and bring more blood and tissue lymph to the part. The blood and tissue lymph contain the immunising and curative properties. It seems to us a simple and convenient way of applying a "blister" to the respiratory tract.

This research has been carried out with the aid of a grant from the London Hospital Research Fund.

[*Note added November* 21, 1911.—We have found that exposure for 10 minutes to two parts in 10 millions of ozone may lower the rectal temperature of rats as much as three degrees, while control rats maintained their normal temperature of 38·5° C.]

# *On the Factors Concerned in Agglutination.*

By H. R. Dean, M.A., M.B., Ch.B., M.R.C.P., Assistant Bacteriologist, Lister Institute, London.

(Communicated by Dr. C. J. Martin, F.R.S. Received October 13,—Read December 7, 1911.)

Of the various reactions which can be observed to take place between antigen and antibody, agglutination has usually been looked upon as relatively simple. It has been assumed that the clumping of bacteria or red cells is produced by the action of substances known as agglutinins. According to the well-known views of Ehrlich, an agglutinin is possessed of two groups, a cytophile or haptophore group which fixes on to the cell, and a group which has the property of producing agglutination. According to another view the cell or bacterium combines with its specific antibody, and the combination of cell and antibody is then clumped by the action of electrolytes. A broad distinction has, however, always been drawn between such phenomena as precipitation and agglutination, which appear to represent a comparatively simple reaction between antigen and antibody, and those more complex effects such as hæmolysis and bacteriolysis in which another constituent of serum, the alexine or complement, is necessary to complete the specific action of the antibody.

Observations published by Muir and Browning (1906) suggested, however, that in some instances, at any rate, the mechanism of agglutination may be more complex.

It was found that fresh ox serum powerfully agglutinated a suspension of ox corpuscles in the presence of antiserum obtained from a rabbit which had been injected with ox corpuscles. The fresh serum could augment to a very marked degree the agglutinative properties of the homologous antiserum. This agglutination of red cells by immune body and complement took place rapidly at 37° C., and somewhat more slowly at room temperature; at 0° C. the agglutination was imperfect. In another experiment, however, complete agglutination was obtained at 0° C. by mixing the red corpuscles of a guinea-pig, the homologous antiserum derived from a rabbit, and fresh ox serum. The property possessed by ox serum of furthering agglutination was destroyed by heating the serum for an hour at 55° C.

In the same year Bordet and Gay (1906) gave an account of a series of very similar experiments. Bordet and Gay independently discovered the agglutinative property of ox serum for sensitised red cells. They found, moreover, that, although this agglutinative property was lost by heating the ox serum at 56° C., it could be restored by adding a little of the fresh serum (complement) of another animal. They concluded that there exists in ox serum a special substance which resists a temperature of 56° C., and which may be preserved for many months in the heated serum. This substance, which was presumably of an albuminous or colloidal nature, showed no tendency to unite with normal corpuscles, but was precipitated on the corpuscles charged with the substance sensibilisatrice and alexine. They called this substance *Colloide du bœuf.* This form of agglutination, which has received the name of conglutination, is attributed by Bordet to the action of three factors on the red corpuscles, namely: (1) the specific antiserum, (2) the ox colloid, (3) a fresh serum or alexine. The heat-resisting substance present in ox serum is called in subsequent papers conglutinin.

Bordet and Streng (1909) published a series of experiments dealing with the agglutinative properties of ox serum. They declared that the conglutinins were essentially different from the agglutinins. As a point of difference they stated that the conglutinins had no need to be fixed on the cells which they conglutinated. This statement appears to be at variance with the previously-quoted observation that the specific substance of the ox colloid is precipitated on corpuscles duly laden with substance sensibilisatrice and alexine.

Bordet and Streng also subjected ox serum after it had been heated to 56° C. to dialysis. They found that the fraction which remained in solution favoured hæmolysis, while the precipitate, if re-dissolved in normal saline, favoured agglutination.

In another communication Streng (1909) claims considerable success for the conglutination method in the identification and differentiation of bacteria. By the addition of ox serum and complement he obtained marked agglutination of bacteria with a dilution of homologous antiserum, which by itself was too weak to produce any trace of agglutination. Streng also stated that conglutinin could be separated from agglutinin by dialysis. The agglutinin, under these conditions, remained in solution, while the conglutinin was precipitated with the globulin fraction.

Barikine (1910) effected a similar separation of agglutinin and conglutinin by saturating ox serum with carbon dioxide. As in the dialysis experiment, the conglutinin was precipitated with the globulin, and the agglutinin remained in solution. Barikine also found that the flocculi of a specific precipitate, formed by the union of antigen and antibody, could be conglutinated by the addition of fresh serum (complement) and heated ox serum (conglutinin).

Bordet and Gengou (1911) published a paper dealing with a phenomenon which they have named co-agglutination, and which they have expressly stated is to be clearly distinguished from conglutination. They found that a mixture of antigen and antibody is able to produce a very marked agglutination of the red cells of a third animal, the guinea-pig. The conditions under which this co-agglutination occurs are carefully set forth by the authors. Guinea-pig blood was found to be the only sort of blood which gave satisfactory results. Defibrinated guinea-pig blood was, as a rule, employed, but equally satisfactory results could be obtained by the use of a suspension of washed corpuscles. Both the serum which contained the antigen and the serum which contained the antibody were heated to 56° C. before use. The co-agglutination was obtained with all the antigen-antibody systems used by the authors. As the co-agglutination was obtained by the use of heated sera and washed guinea-pig corpuscles, the participation of complement could be excluded. Co-agglutination only occurred if a considerable excess of antigen relative to antibody was present in the mixture. The proportions most favourable to co-agglutination were such that the antigen was present in such excess as to inhibit the formation of a precipitate. When the proportions were such that a marked precipitate formed, co-agglutination did not occur. The co-agglutination was not accompanied by any marked fixation of complement. It was necessary that the guinea-pig corpuscles should be present at the time when the antigen came into contact with the antibody. To produce this result the corpuscles were mixed with the antigen and the antibody was then added. Under the proper conditions the agglutination of the red corpuscles was not only very marked but very persistent, that is to say, the corpuscles could be shaken up an indefinite number of times and invariably re-agglutinated.

It would thus appear that the clumping or agglutination of red cells may take place under three different sets of conditions :—

(1) *Agglutination.*—By this is meant the well-known clumping of red cells by a specific antiserum or by a normal serum which possesses agglutinins for the red cells employed.

(2) *Conglutination.*—This is produced by the action of ox colloid (conglutinin) on cells which have been treated with homologous antiserum and with complement. In place of a serum prepared by the injection of an animal with red cells, a normal serum which contains a normal agglutinin for the red cells can, however, be employed.

(3) *Co-agglutination.*—An antigen and homologous antibody can under appropriate conditions agglutinate the red cells of another animal (preferably a guinea-pig).

As regards conglutination and agglutination it appears that the action of ox colloid and complement is to intensify the effect of an agglutinin present in a normal or an immune serum, such action being of the nature of complementing. It is necessary for conglutination that the cells should be sensitised. Muir and Browning, in fact, expressed the view that the ox serum acted as a complement to the immune serum.

The interaction of the various factors in agglutination and conglutination finds to some extent a parallel in phagocytosis, in which the action of a heated serum is intensified by the addition of complement.

### *General Object of the Experiments.*

The experiments here recorded were undertaken as the result of a chance observation. A number of experiments had been made with the view of ascertaining the relative quantities of the two fractions of complement necessary for the production of hæmolysis. In such an experiment it is, of course, essential to put up control tubes which contain the various dilutions of the middle-piece and of the end-piece in order to make certain that neither fraction acting by itself can produce hæmolysis. It was noticed that the corpuscles in the middle-piece control tubes presented a remarkable appearance. Instead of settling down to form a small mass at the very bottom of the tube, the corpuscles were found to be arranged in a thin layer which coated the bottom end and lower part of the tube. The layer of corpuscles took the shape of the lower part of the tube and produced the appearance of a small cup. If the tube was sharply shaken it could be seen that the corpuscles had been agglutinated. The control tube which contained corpuscles and immune body without the middle-piece solution showed no agglutination. The agglutination had been produced by the joint action of the inactivated hæmolytic serum and middle-piece solution.

### *Method of Preparation of Experimental Material.*

*Preparation of Complement Fractions*—

In the experiments which are to be described the complement fractions have been obtained by the carbon dioxide method of Liefmann (1909). Fresh guinea-pig serum is diluted with distilled water in the proportion of one part of serum to nine parts of distilled water. The mixture should be kept cold in an ice bath, and it is an advantage to prepare the mixture with ice-cold distilled water. The mixture is saturated with carbon dioxide and then allowed to stand for one hour in the cold room. The carbon dioxide produces a marked turbidity in the mixture, and at the end of the hour's standing small flocculi are apparent. The precipitate is brought down with a centrifuge and resuspended in ice-cold distilled water. This process of washing the precipitate is repeated, and the precipitate is then dissolved in cold 0·85 per cent. sodium choride solution. The experiments were performed during the summer months, and it was found that, unless care was taken to keep the original mixture and the suspension of precipitate as cold as possible, it was very difficult to redissolve the precipitate in the saline solution. The saline solution was used in such quantity that the resulting middle-piece solution corresponded to a 1 in 10 dilution of fresh guinea-pig serum. The supernatant fluid from which the precipitate had been removed was, as a rule, quite clear, but was generally filtered through filter paper to remove any trace of suspended particles. Sufficient sodium chloride was then added to make it equal to a 0·85 per cent. sodium chloride solution. The resulting solution which contained the end-piece fraction corresponded to a 1 in 10 dilution of the fresh guinea-pig serum.

*Preparation of Other Materials*—

The red corpuscles were obtained from the sheep. The blood was defibrinated with glass beads. The corpuscles were freed from serum by three washings with normal saline solution. The hæmolytic serum was obtained from a rabbit which had had three intravenous injections of washed sheep corpuscles. The serum was inactivated by heating for half an hour at 56° C.

The bacterial emulsions were prepared by emulsifying in normal saline a 24-hours agar culture of *B. typhosus*. The antityphoid serum was obtained from rabbits which had been immunised by intravenous injections of saline emulsions of *B. typhosus* (killed by heating for one hour at 60° C.).

The antityphoid serum was inactivated by heating for half an hour at 56° C.

### *Detailed Description of Experiments and Results.*

*Influence of the Middle-Piece on Agglutination*—

An hæmolytic serum, produced by injecting a rabbit with the washed red corpuscles of a sheep, possesses, in addition to its hæmolytic properties, considerable power of producing agglutination. Agglutination of the red cells is, however, evident only if a rather large amount of antiserum be present in the mixture. In the case of the serum with which these experiments was performed it was necessary to employ the serum in a strength of at least 1 in 100 to obtain any marked degree of agglutination within a period of one hour. If the serum was employed in a dilution of 1 in 200 agglutination could not be detected. It may be mentioned that the hæmolytic titre of this serum was about 1 in 1000. If to a mixture of one volume of a

1 in 20 suspension of red cells, with one volume of a 1 in 200 dilution of heated antiserum, was added one volume of 1 in 10 middle-piece solution, an almost instantaneous and very marked agglutination of the red cells took place. On slanting the tube the red cells could be seen to be aggregated in large clumps. The clumps rapidly increased in size, and after 20 minutes to half an hour had settled to the bottom of the tube to form a single mass which somewhat resembled a soft clot. The supernatant fluid was left quite clear. The viscous mass which formed at the bottom of the tube could be disintegrated by vigorous shaking, but rapidly re-formed, and the process of shaking the clump apart and allowing it to re-form could be repeated indefinitely. One series of tubes was preserved for 48 hours without any change in the condition of the corpuscles. The appearances presented corresponded closely to the description recently given by Bordet and Gengou (1911) of the phenomenon which they called co-agglutination.

It must be plainly stated that the action of the solution of middle-piece is to accentuate the feebly agglutinative action of a small amount of specific antiserum. An agglutination quite as marked and apparently identical in nature could be produced by using a larger quantity of the antiserum without the addition of middle-piece. The action of middle-piece appeared

Table I.

| | 1 c.c. of dilution of hæmolytic serum, Rabbit *v.* Sheep, | + 1 c.c. middle-piece solution diluted. | | | | | + 1 c.c. normal saline. |
|---|---|---|---|---|---|---|---|
| | | 1—10. | 1—20. | 1—40. | 1—80. | 1—160. | |
| 1 | 1—10 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + + |
| 2 | 1—20 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + + |
| 3 | 1—40 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + + |
| 4 | 1—80 | + + + + | + + + + | + + + + | + + + | + + + | + + |
| 5 | 1—160 | + + + + | + + + + | + + + + | + + + | + | + |
| 6 | 1—320 | + + + + | + + + + | + + | + + | 0 | 0 |
| 7 | 1—640 | + + + | + + | + | 0 | 0 | 0 |
| 8 | 1—1280 | + + + | + | 0 | 0 | 0 | 0 |
| 9 | 1 c.c. normal saline | 0 | 0 | 0 | 0 | 0 | 0 |

Each tube contained a volume of 3 c.c. made up of 1 c.c. of a 1 in 20 suspension of washed red cells of the sheep, 1 c.c. of a dilution of heated hæmolytic serum (rabbit and sheep), and 1 c.c. of the diluted middle-piece solution. The tubes numbered 9, in each row, contained no immune serum and the bulk was made up to 3 c.c. by the addition of 1 c.c. of normal saline solution. In these tubes no agglutination occurred, the middle-piece solution by itself being unable to agglutinate the red cells. The tubes in the last column contained 1 c.c. of the suspension of red cells, 1 c.c. of a dilution of the hæmolytic serum and 1 c.c. of normal saline solution. The agglutinative power of the immune body acting by itself is shown in this column. In the remaining columns is shown the effect of the combined action of the immune body and the middle-piece solution.

to be to enormously increase the action of a dilution of antiserum, which if acting by itself would have produced a hardly perceptible degree of agglutination.

The solution of middle-piece was shown by repeated experiments to have no agglutinative action on the red corpuscles in the absence of the antiserum. It is evident from Table I that a very marked degree of agglutination may be produced by the interaction of three components—the red cells, the heated homologous antiserum, and the solution of middle-piece.

### *Remarks on Table I.*

From a consideration of Table I it appears probable that for the agglutination of the red cells two substances are necessary, the one being the specific antibody to the red cells and the other a non-specific substance. Both of these substances are thermostable, and are present in inactivated hæmolytic serum. The larger quantities of the antiserum contained, in addition to the specific antibody, a sufficient quantity of the non-specific substance to produce agglutination of the red cells. If a smaller quantity of the antiserum was used, the amount of non-specific substance was insufficient. In such cases the necessary substance could be supplied by the addition of the solution of middle-piece. An effect of this type is illustrated in Table II.

In this experiment an amount of antiserum was employed which, acting by itself, was unable to agglutinate the quantity of red cells present. Such a quantity of antiserum was found to be 1 c.c. of a 1 in 200 dilution.

Table II.

| | 1 c.c. of dilutions of middle-piece solution. | 1 c.c. of 1—200 dilution of antiserum. | 1 c.c. of 1—20 suspension washed red cells. | |
|---|---|---|---|---|
| 1 | 1—10 | 1—200 | 1—20 | + + + + |
| 2 | 1—20 | ,, | ,, | + + + + |
| 3 | 1—40 | ,, | ,, | + + + + |
| 4 | 1—80 | ,, | ,, | + + + + |
| 5 | 1—160 | ,, | ,, | + + + |
| 6 | 1—10 | 1 c.c. normal saline | ,, | 0 |
| 7 | 1 c.c. normal saline | 1 c.c. 1—200 | ,, | 0 |

Neither the antiserum alone, in a 1 in 200 dilution, nor the solution of middle-piece was able by itself to agglutinate the red cells. The two factors in combination produced a very marked degree of agglutination.

### *Experiment to Show that the Agglutinative Properties of the Middle-Piece Solution are Thermostable.*

The explanation offered in the preceding paragraph assumes that the agglutinating power of an immune serum depends on the presence of two thermostable substances, namely, the specific antibody and a non-specific substance. It is suggested that a deficiency of the non-specific substance in a greatly diluted antiserum may be made good by the addition of middle-piece solution.

Before this explanation can be accepted it is necessary to show that this substance or property of the solution of middle-piece is thermostable, that is to say, capable of resisting a temperature of 56° C. for half an hour. The result of an experiment intended to settle this point is given in Table III.

Table III.

| | Dilutions of the middle-piece solution. | Fresh solution. | Middle-piece solution previously heated at 56° C. | | | |
|---|---|---|---|---|---|---|
| | | | ½ hour. | 1 hour. | 2 hours. | 4 hours. |
| 1 | 1—10 | + + + + | + + + + | + + + + | + + + + | + + + + |
| 2 | 1—20 | + + + + | + + + + | + + + + | + + + + | + + + + |
| 3 | 1—40 | + + + + | + + + + | + + + | + + + | + + + |
| 4 | 1—80 | + + + + | + + + + | + + + | + + | + + |
| 5 | 1—160 | + + | + + | + + | + | + |

Quantities of 5 c.c. of the middle-piece solution were heated at 56° C. for ½, 1, 2, and 4 hours. Parallel dilutions of each sample were then made, and of the original unheated solution. To each tube, which contained 1 c.c. of diluted middle-piece solution, was added 1 c.c. of sheep cells 1 in 20, and 1 c.c. of a 1 in 200 dilution of hæmolytic serum. Control tubes were put up which showed that neither a 1 in 200 dilution of hæmolytic serum nor a 1 in 10 dilution of middle-piece was capable, when acting by itself, of agglutinating the red cells.

### *Remarks on Tables III and IV.*

From this and similar experiments, it was determined that the capacity of the solution of middle-piece to aid in agglutination was only very gradually destroyed at a temperature of 56° C.; heating for half-an-hour had a very slight, or no effect at all, in reducing its activity.

This property of aiding in agglutination can be classed as one of the relatively thermostable properties of serum. It is equally evident that this property of the solution of middle-piece has no connection with its hæmolytic property, for the latter is rapidly lost by subjecting such a solution to a temperature of 56° C. The middle-piece fraction of the hæmolytic complement is definitely thermolabile. On the other hand, whole guinea-pig serum, which had been inactivated in the ordinary way by heating it for half-an-hour at 56° C., was found to possess to a marked degree the property of increasing agglutination. As far as agglutination was concerned, the heated whole guinea-pig serum appeared to possess the same properties as the saline solution of middle-piece.

Table IV.—Comparison of Heated Whole Serum with the Middle-Piece Fraction.

The fresh guinea-pig serum was previously heated for half an hour at a temperature of 56° C.

| | Dilutions. | Heated whole serum. | Middle-piece solution. |
|---|---|---|---|
| 1 | 1—10 | + + + + | + + + + |
| 2 | 1—20 | + + + + | + + + + |
| 3 | 1—40 | + + + + | + + + + |
| 4 | 1—80 | + + + | + + + + |
| 5 | 1—160 | + + | + + |

The two sets of dilutions were comparable, that is to say, the 1 in 10 dilution of middle-piece solution corresponded to the amount of middle-piece in a 1 in 10 dilution of whole serum.

To every tube was added a suspension of red cells and the diluted immune serum. It was shown by appropriate controls that neither this dilution of immune serum, 1 in 200, nor the heated whole serum, nor the middle-piece solution, in the dilutions employed, had the power of agglutinating the red cells.

### *Influence of the End-Piece.*

Only a few experiments were carried out with a view to ascertaining the influence of the end-piece fraction of the complement. In one case the solution of end-piece had no influence on agglutination, acting either alone or in conjunction with the middle-piece solution. Another end-piece solution had a slight influence in a dilution of 1 in 10 in increasing the agglutination of the red cells by an antiserum. When further diluted it had no action. The same solution of end-piece somewhat increased the agglutination produced by the interaction of antibody and middle-piece.

It should be added that neither solution of end-piece had in the dilutions employed the slightest agglutinative action on the red cells in the absence of the specific immune body. The discrepancy between the two solutions probably depended on slight differences in the method of separation.

In any case, the substance which aids in agglutination appears to be contained chiefly in the fraction of the globulin which is precipitated by carbon dioxide. The agglutinating substance is present in whole normal serum, but its agglutinative property can be conveniently studied in a middle-piece solution, since the progress of agglutination is not interrupted by the lysis of the red cells. For this reason a middle-piece solution prepared from fresh guinea-pig serum was used in the majority of the experiments.

### *Addition of Middle-Piece to Sensitised Cells.*

The action of the solution of middle-piece can be demonstrated in a very striking manner by adding middle-piece solution to corpuscles previously sensitised with the homologous antiserum.

One cubic centimetre of a 1 in 20 suspension of sheep red cells was added to 1 c.c. of a 1 in 200 dilution of antiserum. The mixture was allowed to stand for half-an-hour at room temperature. At the end of this time there was no evidence of agglutination. There was then added 1 c.c. of a 1 in 10 solution of middle-piece. The red cells immediately ran together into large clumps and rapidly settled to the bottom of the tube. This experiment showed that the middle-piece solution could exert its action on already sensitised red cells, and that it was not essential that the middle-piece should be present from the time of the first admixture of antigen and antibody. The sensitised red cells can, in fact, if freed by repeated washings from every trace of uncombined antibody, be still agglutinated by the addition of middle-piece solution. Previously sensitised red cells are, in fact, agglutinated with great rapidity, for time is not taken up by the union of the red cells with the antibody.

### *Influence of Temperature on the Reaction.*

No strictly comparative experiments have as yet been undertaken with a view to ascertaining the influence of temperature on the agglutination of red cells by immune body and middle-piece solution. The majority of experiments were carried out at room temperature, but the agglutination was somewhat accelerated by placing the tubes in an incubator at 37° C. On the other hand, it was ascertained that a very marked degree of agglutination was reached when the tubes were placed in the cold room at a temperature of a few degrees above 0° C. The middle-piece solution is certainly able to agglutinate sensitised corpuscles in the cold, and the delay in agglutination is sufficiently explained by the longer time required at a low temperature for the union of the red cells with antibody.

### *Agglutination of Bacteria.*

A considerable number of experiments were made with the object of reproducing with bacterial emulsions the results obtained by the use of blood corpuscles. In these experiments an inactivated antityphoid serum derived from a rabbit and an emulsion in normal saline of a 24-hours' agar culture of *B. typhosus* were used. Such an experiment is represented in Table V.

Table V.

| | 0·5 c.c. of inactivated diluted antityphoid serum. | A.<br>+1 c.c. normal saline. | B.<br>+0·5 c.c. middle-piece 1—10, and 0·5 c.c. normal saline. | C.<br>+0·5 c.c. middle-piece 1—10, and 0·5 c.c. end-piece 1—10. |
|---|---|---|---|---|
| 1 | 1 in 4000 | + + | + + + | + + + |
| 2 | 1 in 5000 | + | + + + | + + + |
| 3 | 1 in 6000 | 0 | + + | + + |
| 4 | 1 in 7000 | 0 | + + | + + |
| 5 | 1 in 8000 | 0 | + | + |
| 6 | 1 in 9000 | 0 | 0 | 0 |
| | Controls | | | |
| 7 | 0·5 c.c. normal saline | 0 | 0 | 0 |

Every tube contained 2 c.c. In the control tubes the bulk was made up to 2 c.c. with normal saline. Tube 7 contained in column A 0·5 c.c. of bacillary emulsion and 1·5 c.c. of saline; in row B emulsion and middle-piece; in row C emulsion and middle-piece and end-piece. In these controls no agglutination was observed. In row A the agglutinative power of the immune serum is recorded. No agglutination was observed below tube 2. It will be seen that this tube contained 0·5 c.c. of emulsion of bacteria, 0·5 c.c. of a 1 in 5000 dilution of antityphoid serum, and 1 c.c. of normal saline solution. The ultimate dilution of the immune serum was in consequence 1 in 20,000. Column B shows the agglutination produced by immune serum in the same dilutions plus 0·5 c.c. of a 1 in 10 dilution of middle-piece in every tube. All the tubes were incubated for 4 hours at 37° C.

### *Remarks on Table V.*

It will be seen that the agglutinative power of the immune serum is increased by the addition of middle-piece. The result of the addition of end-piece to middle-piece and immune serum is shown in Column C. No further increase in agglutination was observed. Numerous experiments were made which gave results consistent with those shown in Table V.

To demonstrate the effect of middle-piece solution it is necessary to dilute the immune serum to such an extent that its agglutinative power is just beginning to disappear. The action of such diluted antiserum can be increased by the addition of middle-piece solution. Further, the agglutinative power of an antiserum which has been diluted beyond the point at which the agglutination can be appreciated, can be restored by the addition of the middle-piece solution.

These experiments show that there exists in normal guinea-pig serum some substance which increases the agglutinative action of antiserum. This substance is thermostable, and probably has no connection with complement. It can be separated from serum with the fraction of the globulins which are precipitated by diluting a serum with distilled water and saturating the mixture with carbonic acid. In these characteristics it conforms to the description of conglutinin which is given by Bordet and Streng. The substance may be neither more nor less than serum globulin or some fraction of globulin which is easily precipitated.

The experiments suggest that an agglutinating serum contains a specific antibody and a non-specific substance, both of which are necessary to produce agglutination. When such a serum is greatly diluted the dilution may contain sufficient of the specific antibody but not sufficient of the non-specific anti-substance. In such a case the non-specific substance can be supplied by adding middle-piece solution prepared from normal serum, and agglutination is produced.

### *Experiments to Determine the Way in which the Middle-Piece Solution aids in Agglutination.*

The inter-relation of the factors concerned in agglutination is to some extent illustrated by the following experiment. Ten cubic centimetres of a 1 in 20 suspension of washed sheep red corpuscles were mixed with 20 c.c. of a 1 in 200 dilution of a heated hæmolytic antiserum. The mixture was allowed to remain in the cold room for one hour and was then centrifugalised. The corpuscles were then thoroughly washed and again centrifugalised. The sensitised corpuscles were then mixed with 10 c.c. of middle-piece solution. In another tube an equal quantity of unsensitised sheep corpuscles was added to 10 c.c. of middle-piece solution. Both tubes were placed in the cold room. At the end of this time very marked agglutination had taken place in the tube containing the sensitised corpuscles. The contents of both tubes were centrifugalised. The deposit of agglutinated cells was shaken up in normal saline and again centrifugalised. After two washings the deposit was thoroughly shaken up and suspended in normal saline. Within 15 minutes the cells had re-agglutinated, and after half an hour had fallen in a compact mass to the bottom of the tube.

The supernatant fluid from the tube which contained the sensitised red cells was added to a further quantity of sensitised red cells. After the lapse of several hours a very slight degree of agglutination could be detected. It is evident that sensitised red cells can, in the process of agglutination, remove from the solution of middle-piece the substance which produces the agglutination. The supernatant fluid from the tube which had contained the unsensitised red cells contained the agglutinative substance in a state of unimpaired activity. Some substance had been taken out of solution by the sensitised but not by the unsensitised cells.

The above experiment shows that sensitised cells remove from a middle-piece solution the substance which causes their agglutination. To demonstrate the manner in which this substance was removed it was decided to employ a solution of the constituents of the corpuscles in distilled water. One cubic centimetre of thoroughly washed sheep corpuscles was laked with 9 c.c. of distilled water. The solution of corpuscles was made up to the usual saline content by the addition of 10 c.c. of 1·7 per cent. sodium chloride solution. After filtering many times through filter paper a perfectly clear solution was obtained, representing a 1 in 20 solution of red corpuscles in normal saline.

The following mixtures were then prepared :—

| Tube. | Solution of laked corpuscles. | Antiserum, rabbit *v.* sheep cells, 1 in 200. | Normal saline solution. | Solution of middle-piece 1 in 10. | Normal saline solution. |
|---|---|---|---|---|---|
| | c.c. | c.c. | c.c. | c.c. | c.c. |
| 1 | 5 | 5 | — | 10 | — |
| 2 | 5 | — | 5 | 10 | — |
| 3 | 5 | 5 | — | — | 10 |
| 4 | — | 5 | 5 | 10 | — |

In tube 1 5 c.c. of the solution of corpuscles was added to 5 c.c. of the diluted antiserum. In the control tubes 2, 3, and 4 the ingredients indicated in the first three columns were mixed. The volume in each tube was then 10 c.c. All the tubes were allowed to remain for one hour at room temperature. They were then examined and the contents were found to be absolutely clear. To tubes 1, 2, and 4 were then added 10 c.c. of the 1 in 10 middle-piece solution; to tube 3 was added 10 c.c. of normal saline solution. All four tubes were then incubated for four hours at 37° C., and then placed for 12 hours in a cool chamber at about 8° C. Tube 1 was then found to contain a small but definite white flocculent deposit. The three control tubes remained absolutely clear.

This experiment with unimportant variations in detail was several times repeated. In every case the tube which contained antigen, antibody, and middle-piece solution contained a precipitate. The control tubes contained no precipitate.

### *Results obtained by adding Middle-Piece Solution to a Mixture of a Normal Serum with its Homologous Antiserum.*

It has been shown that sensitised red corpuscles are agglutinated by middle-piece and that in the process of agglutination the substance which produces the agglutination is removed from solution. It has also been shown that a precipitate is formed in a mixture of laked corpuscles, antibody, and middle-piece. This suggests that the substance present in the middle-piece solution is actually precipitated on the sensitised corpuscles and that such precipitation is a part of the mechanism of agglutination.

It was decided to examine the effect of adding middle-piece solution to a

Table VI.

| | 3 c.c. of horse serum (antigen) diluted. | + 3 c.c. antiserum, rabbit *v.* horse, diluted 1 in 10. | | + 3 c.c. antiserum, rabbit *v.* horse, diluted 1 in 100. | | + 3 c.c. normal saline solution. | |
|---|---|---|---|---|---|---|---|
| | | (A) + 3 c.c. normal saline. | (*a*) + 3 c.c. middle-piece 1 in 10. | (B) + 3 c.c. normal saline. | (*b*) + 3 c.c. middle-piece 1 in 10. | (C) + 3 c.c. normal saline. | (*c*) + 3 c.c. middle-piece 1 in 10. |
| 1 | 1 in 5 | Large precipitate | Large precipitate | Clear | Clear | Clear | Clear |
| 2 | 1 in 10 | ” | ” | Very slight opalescence | Opalescence | ” | ” |
| 3 | 1 in 20 | ” | ” | ” | Marked turbidity | ” | ” |
| 4 | 1 in 40 | ” | ” | Clear | ” | ” | ” |
| 5 | 1 in 80 | ” | ” | ” | Opalescence | ” | ” |
| 6 | 1 in 160 | ” | ” | ” | Slight opalescence | ” | ” |
| 7 | 1 in 320 | Precipitate | Precipitate | ” | Clear | ” | ” |
| 8 | 1 in 640 | ” | ” | ” | ” | ” | ” |
| 9 | 1 in 1280 | Turbidity | Turbidity | ” | ” | ” | ” |
| 10 | Controls. 3 c.c. saline solution | Clear | Clear | Clear | Clear | Clear | Clear |

All the tubes contained 9 c.c. The tubes numbered 1 to 9 in each column contained various dilutions of normal horse serum (antigen). To the tubes in column A were added 3 c.c. of normal saline solution and 3 c.c. of antiserum in a dilution of 1 in 10. To the tubes in row *a* were added 3 c.c. of a middle-piece solution (1 in 10) and 3 c.c. of antiserum 1 in 10. A bulky precipitate was formed in the tubes of column A and of column *a*. There was no difference in the appearance of the tubes in column A and in the tubes in column *a*. To the tubes in column B were added 3 c.c. of normal saline and 3 c.c. of antiserum diluted 1 in 100. To the tubes in column *b* were added 3 c.c. of middle-piece solution 1 in 10 and 3 c.c. of antiserum diluted 1 in 100. This dilution of antiserum produced a rather doubtful opalescence in tubes 2 and 3 of column B. The remaining tubes of column B were clear. A very distinct difference existed between the tubes in column B and the tubes in column *b*. In the tubes in column *b* opalescence or turbidity was distinctly visible in tubes 2 to 6. The tubes in column C contained horse serum only. The tubes in column *c* contained horse serum and middle-piece. No precipitate was produced by this mixture. The tubes marked 10 in the various columns show that neither antiserum alone nor antiserum and middle-piece solution produced a precipitate.

It will be noticed that the tubes 1 in columns B and *b* showed no opalescence. This inhibitory effect is due to relative excess of antigen.

The tubes were incubated for 4 hours at 37° C., and then allowed to stand overnight in the cold room.

mixture of normal horse serum and the serum of a rabbit which had been injected with horse serum. Both the horse serum and the antiserum were heated for half an hour at 56° C. before use. Since such a mixture of serum and antiserum produces a bulky precipitate it was found necessary to dilute the antiserum to such an extent that only a slight trace of opalescence was produced when it was mixed with the antigen. The antiserum used was found to give a hardly perceptible opalescence in a dilution of 1 in 100. If less diluted antiserum was employed the precipitate formed was so large as to make it impossible to determine if the middle-piece solution took any part in the reaction. The result of such an experiment is shown in Table VI.

### *Remarks on Table VI.*

From a consideration of Table VI it appears that a mixture of a normal serum with its homologous antiserum is able to effect the precipitation of some substance present in the middle-piece solution. This effect can only be demonstrated by using a greatly diluted antiserum. As in the agglutination experiments the effect of adding middle-piece solution was only demonstrable if it was added to a small quantity of antiserum. It also appeared that for the effective precipitation of middle-piece it was necessary that there should be present a relative excess of antigen. This is of interest since it is precisely under these conditions that Bordet was able to produce the phenomenon which he called co-agglutination.

### *On the Properties of the Globulin Solution prepared from Sheep Serum.*

The capacity of the middle-piece or globulin solution obtained from guinea-pig serum does not appear to be a peculiarity of the serum of the guinea-pig. Fresh sheep serum was treated with $CO_2$ in a similar fashion and the resulting solution of the globulin precipitate had the power of increasing agglutination in a suitable mixture of sheep corpuscles and hæmolytic antiserum. The result of adding a solution of sheep globulin to a mixture of horse serum with rabbit *v.* horse serum is shown in Table VII.

The following mixtures were prepared :—

Table VII.

| | Normal horse serum diluted 1 in 10. | Rabbit *v.* horse serum diluted 1 in 50. | Sheep middle-piece solution 1 in 10. | Normal saline solution. |
|---|---|---|---|---|
| | c.c. | c.c. | c.c. | c.c. |
| 1 | 5 | 5 | 10 | — |
| 2 | 5 | — | 10 | 5 |
| 3 | — | 5 | 10 | 5 |
| 4 | 5 | 5 | — | 10 |

All four tubes were incubated for six hours at 37° C. and then allowed to stand for 12 hours in the cold room. A distinct turbidity formed in tube 1. The contents of the remaining three tubes remained perfectly clear.

The middle-piece or globulin solution obtained from sheep serum was shown to have the same properties as the middle-piece solution obtained from guinea-pig serum. It is proposed to supplement these experiments by examining the properties of the globulin solutions of a variety of animals.

### *Discussion of Results.*

An agglutinating serum contains two factors, both of which are necessary to agglutination. The one is the specific antibody, the other a precipitable substance, probably of the nature of a globulin. By the interaction of antigen and antibody the molecules of the precipitable substance are aggregated on the surface of the blood corpuscle or bacterium which is to be agglutinated.

The amount of specific antibody necessary to produce agglutination is probably minute, and, by diluting an antiserum, a dilution can be obtained which contains sufficient antibody but not sufficient of the precipitable substance. By adding to such a dilution of the antiserum a solution of the precipitable substance, derived from normal guinea-pig serum, agglutination can be effected. The amount of precipitable substance necessary to produce the agglutination of sheep corpuscles appears to be considerably larger than the amount required to agglutinate typhoid bacilli. The precipitable substance is thermostable, it is present in heated normal serum, and it can be precipitated from normal serum with a fraction of the serum globulin. It can also be precipitated from a solution in normal saline by a suitable combination of an antigen with its antibody. This precipitate is small and does not become visible until the experiment has been incubated for several hours.

It is probable that agglutination is effected during the earlier stages of the aggregation of the molecules of the precipitable substance, that is to say, before the process has advanced to the stage when a turbidity is visible. The precipitable substance is probably identical with "conglutinin." There is, however, this difference between the results obtained in these experiments and the conglutination effects of Bordet and his collaborators.

In Bordet's experiments, conglutination was obtained by the interaction of four factors, namely, the red cells, the heated antiserum, heated ox serum (conglutinin), and complement. In the experiments described in this paper, agglutination was effected by the interaction of three factors—the red cells the heated antiserum, and the substance present in the middle-piece fraction of guinea-pig complement.

Sufficient experiments have not been performed to justify a definite statement as to the relation of the phenomena of conglutination and agglutination. Nevertheless, it seems possible that agglutination and conglutination are essentially the same process. This process is the aggregation or precipitation of a precipitable substance by the interaction of antigen and antibody. In the case of agglutination this substance is a constituent of the agglutinating serum. In the case of conglutination a further supply of this substance is supplied from another source (ox serum).

The phenomenon described under the name of co-agglutination is of great interest in that the antigen is not a constituent of the agglutinated cell, but is derived from some different source. In such an experiment the interaction of antigen with antibody produces such a change in the physical conditions of the mixture that the suspended corpuscles, which may be supposed to have no affinity for the antigen or antibody, are spontaneously agglutinated. The result suggests the possibility that in an ordinary agglutination experiment the corpuscles may be agglutinated as the result of a reaction between antibody and antigen, which has diffused out of the corpuscle into the surrounding fluid. If such a view be correct, it follows that the phenomena described as agglutination, conglutination, and co-agglutination are essentially the same.

Apart from the question of agglutination, the results recorded may possibly be found to have some bearing on other serum reactions. The influence of the middle-piece and end-piece fractions of the complement in phagocytosis has been investigated by Dr. Ledingham in conjunction with the author, and the results of these experiments are shortly to be published.

With regard to the formation of precipitates, the experiments suggest that a suitable mixture of serum and antiserum is capable of precipitating a non-specific substance derived from the serum of a third animal. It seems, indeed, probable that the reason why an antiserum, if diluted, loses its power of producing a precipitate is not because the dilution contains too little antibody, but because there is not sufficient precipitable substance present to produce a precipitate.

It is sometimes held that, because a mixture of antigen with a dilution of antiserum can be prepared which shows no precipitate and nevertheless efficiently binds complement, the complement-binding antibody must be different from the precipitate-forming antibody. Now it has been shown in Table VI that a mixture of certain proportions of horse serum with its homologous antiserum may remain quite clear, while on the addition of the globulin solution of guinea-pig serum a turbidity appears. Now this globulin solution contains the middle-piece fraction of guinea-pig complement, the fraction which is known to disappear in a complement-fixation experiment. It is proposed, therefore, to make these questions the subject of further investigation.

### *Summary.*

(1) Sheep corpuscles are, as is well known, agglutinated by an homologous antiserum. If, to a mixture of corpuscles with antiserum so dilute that no agglutination is visible, there be added a solution of globulin obtained from normal guinea-pig serum, the corpuscles are markedly agglutinated. By the use of suitable controls it can be demonstrated that neither the globulin solution nor the dilution of antiserum employed are of themselves capable of agglutinating the corpuscles.

(2) The substance present in the globulin solution which aids agglutination is relatively thermostable, and its presence can be demonstrated in whole heated guinea-pig serum.

(3) Corpuscles which have been sensitised and washed to remove free antibody can be agglutinated by the globulin solution. If, after agglutination has taken place, the corpuscles be removed with a centrifuge, the supernatant fluid can be shown to have lost its agglutinating property.

(4) The agglutinating power of an extremely dilute antityphoid serum can be increased by the addition of the globulin solution. By the addition of globulin solution to a mixture of emulsion of *B. typhosus* with a dilution of antiserum which is too weak by itself to agglutinate the bacilli, distinct agglutination can be obtained.

(5) The formation of a specific precipitate by the interaction of a serum with its homologous antiserum depends, as is well known, on the presence in the mixture of a relatively large amount of the antiserum. If, to a mixture of serum with antiserum so diluted that it is no longer able to produce a precipitate, is added the globulin solution, a definite turbidity is produced.

(6) It seems probable that an agglutinating serum (antiserum) contains two factors, both of which are necessary to produce agglutination. The one of these is the specific antibody, the other is a non-specific substance which is possibly serum globulin. The interaction of antigen with antibody produces an aggregation of the molecules of the non-specific substance which may ultimately result in the formation of a definite turbidity. This process of aggregation of the particles of the non-specific substance is an essential part of the process of agglutination. It is possible to make a dilution of an antiserum which contains sufficient of the specific anti-substance but not sufficient

of the non-specific substance. The deficiency in non-specific substance can be made up by the addition of a globulin solution obtained from normal serum.

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# *Colour-Blindness and the Trichromatic Theory of Colour Vision.*
## Part III.—*Incomplete Colour-Blindness.*

By Sir W. de W. Abney, K.C.B., F.R.S.

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## *Address of the President, Sir Archibald Geikie, K.C.B., at the Anniversary Meeting on November* 30, 1911.

The first duty which devolves upon us at these Anniversaries is to take note of the losses by death which the Society has suffered during the year that has passed. The sadness which cannot but be felt in recounting these losses and realising by how much poorer they have made the Society is, perhaps, somewhat lessened on the present occasion by the fact that our ranks have suffered rather less diminution than usual. On the Home List we have lost thirteen Fellows, on the Foreign List only one.

At the Anniversary last year, in presenting the Copley Medal, I had an opportunity of briefly referring to some of the leading features in the career of Sir Francis Galton, to whom the Medal had been awarded. Within a few weeks thereafter that distinguished man, full of years and honours, passed to his rest. In the brief interval of these weeks, I had the pleasure of visiting him at his temporary home in the country, and of hearing from his own lips how greatly he was gratified that the Royal Society, of whose Fellowship he was always so appreciative, should have bestowed on him its highest honour. It was, he said, the crowning distinction of his life. I did not think at the time that it would be the last mark of recognition that would come to him, for he looked as well as he had done for a long time; his keen interest in scientific progress was unabated, and his mind and memory clear as ever. In him we mourn an accomplished and generous man of science, who devoted his long life and energies to the advancement of natural knowledge. It is a pleasing remembrance to us that in conferring the Copley Medal upon him the Royal Society brightened the last days of one of the most loyal of its Fellows.

On the side of the physical sciences the Society has lost some prominent representatives. In Dr. Johnstone Stoney another has passed away of that brilliant band of physicists whom Ireland has given to science. He died on July 1 last at the ripe age of 85, carrying with him to the grave the affectionate regrets of a wide circle of friends, who appreciated his scientific labours and lifelong enthusiasm, and who esteemed his gentle and kindly nature.

Samuel Hawksley Burbury, who was a very regular attendant at our meetings, died on August 31, in his 80th year. He had at Cambridge a career which was remarkable for combining the highest honours in classical literature with mathematical distinction. He was called to the Bar in

1858, and, in the midst of his legal work, found time to extend his mathematical studies. He thus became a high authority on the dynamical theory of gases and other branches of physical mathematics.

JOHN BROWN, formerly a linen manufacturer of Belfast, who only died at the beginning of the present month, deserves to be remembered as another representative of the now dwindling class of men of business who devote their leisure to scientific pursuits and the promotion of knowledge. His papers on the seat of the electromotive force in voltaic combinations, especially on the influence of the surrounding medium, contributed substantially to the elucidation of that subject. He became a Fellow of the Society in 1902.

FREDERICK JERVIS-SMITH, formerly Millard Lecturer in Experimental Mechanics at Trinity College, Oxford, and a devoted worker in that subject, was remarkable for his skill in the construction of delicate mechanical appliances in the laboratory which he fitted up in his College. He was elected into the Society in 1894, and died on August 23 last, at the age of 63.

MERVYN HERBERT NEVIL STORY-MASKELYNE was the bearer of a name which is honoured in the history of science and in that of the Royal Society, and which received additional distinction from his own labours. For almost forty years Professor of Mineralogy at Oxford, and for twenty years of that period likewise Keeper of the Department of Minerals in the British Museum, he stood at the head of mineralogical science in this country. By his lectures, his writings, and, above all, by his labours in the augmentation and arrangement of the admirable mineral collection in our National Museum, he did much to encourage the study of mineralogy, which had been somewhat neglected in Britain.

JOHN ATTFIELD will be remembered for the value of his contributions to chemical pharmacology. By his teaching and writings, and his constant personal exertions in raising the standard of education among pharmaceutical chemists, he rendered great service to the branch of applied science which he cultivated. He died on March 18 at the age of 76.

Besides these losses on the Home List from the ranks of our physicists and chemists, we have to record, with sincere regret, the death of one of the most notable of our Foreign Members, the illustrious JACOBUS HENRICUS VAN'T HOFF. His genius, combining a remarkable union of mathematical acumen, experimental resource, and faculty for bold and lofty generalisation, opened up new domains in chemistry. His work on 'Chemistry in Space' laid the foundations of stereo-chemistry, and his 'Studies in Chemical Dynamics' placed that side of the science on a well-established basis. In recent years he has been engaged on a series of elaborate researches into the conditions in which deposits from saline solutions can be formed in the sea. His papers on this subject throw fresh light on the history of accumulations of this nature which are intercalated among the strata of the earth's crust, and his work is thus of interest alike to the chemist and the geologist.

On the side of the biological sciences, six of our Fellows have died during the past year. The cause of research in tropical medicine has suffered a grievous loss by the premature death of SIR RUBERT BOYCE. His career of only forty-eight years has been marked by unwearied energy and enthusiasm in the contest with the malignant diseases that are the scourge of man in tropical climates. Not merely did he personally carry on researches in this country and encourage others to co-operate in the same cause, but, throwing himself into the breach, he again and again sailed to the Tropics for the purpose of enquiring into the maladies on the spot. His labours, and those of the other investigators who have studied yellow fever, have been rewarded, and now that fatal malady has been successfully combated.

Of the physicians on the list of our Fellows we have to record the deaths of three eminent men. JOHN HUGHLINGS JACKSON was the founder of the modern school of neurology in this country. Perhaps his greatest work was his discovery, on purely clinical grounds, of the localisation of function in the centre of the brain—a discovery that has been verified and greatly extended by a long series of experimental researches by other observers.

FREDERICK WILLIAM PAVY, for so many years a familiar figure at our meetings, has passed away in his eighty-third year. He has held a high place among the physicians of his day, not only as an eminent practitioner, but as an accomplished and assiduous man of science, who devoted his long life mainly to one special branch of investigation—the part played by sugar in the economy of the animal system. The important bearing of his investigations on diabetes and other diseases has long been recognised both in this country and abroad.

SIR SAMUEL WILKS was remarkable for the keen insight shown in his recognition of the fact that medicine must rest on the science of pathology. He devoted his life and teaching to the development of this principle. His contributions to pathological knowledge were many and valuable in themselves, but they acquired additional importance from the correlation which he established between the findings of pathology and of morbid anatomy on the one hand, and the natural history of disease, as seen clinically, on the other. To the end of his life he took the greatest and most appreciative interest in the new and striking developments of his own favourite science.

To our late associate, Dr. JOHN BEDDOE, the science of anthropology stands greatly indebted. Born in 1826, and educated for the medical profession, he began, when only 20 years of age, to make those observations on the facial and other features of living races which, throughout his busy professional life, he continued to prosecute till he became the most learned and accomplished authority on the anthropological history of the human races of Britain and of the European Continent.

The name of THOMAS RUPERT JONES has been for nearly two generations a household word among the palæontologists and geologists of this country. Although his own more particular branch of enquiry lay among the Entomostraca and Foraminifera of past ages, on which he was the highest authority, he possessed a wide range of acquirement in all departments of geology. His ample stores of knowledge were always freely placed at the service of other workers in science. Born in 1819, he passed away last spring at the advanced age of 92.

The Report of the Council for the past year, now in the hands of the Fellows, gives a summary of the work on which the Society has been engaged since the last Anniversary. There are one or two features in this Report to which I should like to call attention. In my Address last year I adverted to the history of seismological observation in this country and to the part taken in the development of this branch of observational science by our associate Dr. Milne. I expressed the hope that means might be found to place his important service on a more permanent footing, with an enlarged staff and more generous financial aid. Though no important advance has yet been made towards the realisation of this hope, the subject has not been lost sight of, and at least one useful step has been taken in the more complete equipment of Eskdalemuir Observatory as a seismological station. There are now installed there the complete Galitzin apparatus and the twin Milne apparatus, which record photographically, and also the Wiechert and the Omori instruments, the observations of which are recorded on smoked paper. To Prof. Schuster we are indebted for his generosity in presenting the Galitzin apparatus. The various instruments, when completely put into working order, will supply valuable material for a comparison of results and will provide an important addition to the network of seismological stations in this country. The addition of this seismological work to the other duties of the Superintendent of the Eskdalemuir Observatory has shown that an increase of the staff under his supervision is imperatively required. The Gassiot Committee, after a full consideration of the subject, has recommended that a grant in aid for a limited period should be made by the Royal Society, and the Council, approving of the proposal, has granted a sum of £450 for the purpose of supplying an additional observer for two years, after which some other more permanent arrangement must be provided. In the meantime the Council has been gratified by the gift of £200 from Mr. Matthew Gray for the purpose of assisting the progress of seismology at Eskdalemuir.

Fellows are aware that for many years past the Society has been conducting researches into the cause and prophylactic treatment of tropical diseases, and that these researches are still in progress. Much information has been collected, and it is satisfactory to know that, since steps have been taken to remove the native population from the fly-belts, the areas affected by one of the most terrible of these maladies, Sleeping Sickness, have been considerably restricted. But much remains to be accomplished before the knowledge of the subject can be made as complete as it should be. As will be seen from the Report of the Council, the investigation is now about to be extended far beyond the bounds originally contemplated. It has been plausibly suggested that Sleeping Sickness may be transmitted from other sources than infected human beings, and the question arises whether the wild animals of tropical Africa may possibly supply the trypanosomes of that disease. Accordingly, at the request of the Colonial Office, the Royal Society has organised and despatched a new Commission, under the directorship of Sir David Bruce, for the purpose of studying on the spot what may be the relation of the native fauna of Nyasaland and other parts of Africa to the spread of human trypanosomiasis, and what trypanosome diseases may affect the domestic animals of that region. The composition of the staff has been carefully considered with a view to secure adequate attention to each of the various branches of investigation that are embraced in the wide enquiry which is projected. It is interesting to know that Lady Bruce, who has all along been one of the most efficient observers in Africa, again accompanies her husband on this fresh expedition. I may add that she is not the only lady engaged under our auspices in Africa; Miss Robertson, who has had considerable experience in the study of trypanosomes, has volunteered her services in Uganda, and is now at the Mpumu laboratory, tracking the development and transmission of the organisms to which trypanosomiasis is due.

To what is said in the Council's Report regarding the progress of the National Physical Laboratory I have one important addition to make. The Fellows of the Society who may not have previously heard will now be grieved to hear of the serious illness which last month attacked our esteemed and accomplished colleague, the Director of the Laboratory. After a time of painful suspense Dr. Glazebrook slowly began to recover, and is now happily on the high road to convalescence. But it may be some months before he can again attend to the work of the Institution over which he presides with such constant assiduity and skill.

The 'Catalogue of Scientific Literature for the Nineteenth Century,' on which the Committee of the Royal Society has now been engaged for over fifty years, is speedily approaching completion. The material for the final part (1883—1900) of the General Catalogue, which is classified under authors' names, has been collected and sorted, and is nearly ready to pass through the press. Of the subject-indexes of scientific papers for the nineteenth century, two volumes, Pure Mathematics and Mechanics, have been published; and the Index for Physics, in two volumes, is well under way. While the Committee do not claim perfection in detail for the classification of the subject-matter of those sciences, and while they are aware that the arrangement of so great a mass of material, which must be condensed into small space, will always be liable to technical criticism in details, they nevertheless believe that it may be confidently claimed that no person who in future shall set about a general investigation or an historical survey in any department of one of these sciences can afford to neglect consultation of this index. It was felt to be worth while by so great a man as Thomas Young, a hundred years ago, to devote a large amount of time to the compilation of a classified index of the literature of Natural Philosophy up to that date, when the achievement was just within the range of private enterprise. The immense volume of the scientific literature of the last century could have been digested only by some corporate organisation; and the whole scientific world have signified in advance their obligation to the Committee of the Society and to the generous benefactors who have assisted the Society in the work when its own funds had been depleted, by undertaking the continuation of the same work in the twentieth century as the 'International Catalogue of Scientific Literature.'

Having gone to so much trouble and expense in the preparation of the materials for these subject-indexes, the Society is naturally desirous to see that the results become accessible to the scientific public, for whose use the volumes are intended. All the funds which the Royal Society can possibly devote to this work are necessary for its completion; thus there can be no question of free exchange, as was the case with the earlier volumes, however much the Royal Society might desire it. But, as the Fellows are already aware, the Cambridge University Press have consented to undertake the entire risk of printing and publication, and have agreed to sell the volumes at a very moderate price. We are informed that the volumes of the Index already issued have, for some reason, not yet attracted the attention among Universities and public libraries that was confidently anticipated. I have therefore thought it desirable to bring this matter to notice to-day.

On July 15 of next year the Royal Society will have lived for exactly two centuries and a-half. Looking back upon this long career, and considering the friendly relations which the Society has for generations maintained with the men of science in all quarters of the globe, the President and Council have thought that the occasion will be one which ought not to be passed over in silence, but which deserves to be marked in some worthy way. They have accordingly decided to invite the chief universities, academies, scientific societies, and other institutions in this country, in our Colonial Dominions and abroad, to send delegates hither to join with us in celebrating our 250th birthday. The invitations will be issued next month, so as to allow ample time for the selection and the arrangements of the delegates, and for our own preparations here. Our patron, His Majesty the King, has been pleased to signify his appreciation of the importance of our proposed celebration. Though the details of the function have not yet been settled, it is thought that the first reception and welcoming of our guests should be held in our own rooms, which, with their portraits and other memorials of our past, will doubtless be of interest to the visitors. For the banquet, at which the Fellows and their guests will dine together, we hope to enjoy the use of a large hall specially lent to us for the occasion. Considering the early association of the Royal Society with Gresham College and the City, we trust that some opportunity will be afforded to us of renewing that intercourse, and thus of allowing our delegates to partake of the well-known hospitality of London. There will doubtless be a good deal of private hospitality. Of course, every facility will be arranged for our guests to see public buildings, museums, libraries, and other objects of interest. At the end of the function in London, the delegates may not improbably be invited to visit the Universities of Oxford and Cambridge.

As a permanent memento of the occasion, the Council has decided to reproduce in facsimile the pages of the Charter-book, containing the signatures of the Fellows from that of the founder, Charles II, down to the present day. This interesting volume is now in course of preparation at the Oxford University Press. It has also been arranged to issue a new edition of the Society's 'Record,' in great part re-written, closely revised, and brought up to date. This volume is also in progress.

## MEDALLISTS, 1911.

### THE COPLEY MEDAL.

The Copley Medal is this year awarded to Sir George Howard Darwin for his long series of researches on tidal theory, including its bearing on the physical constitution of the earth and on problems of evolution in the planetary system.

As regards the actual oceanic tides, he has perfected the method of harmonic analysis initiated by Lord Kelvin, and has greatly promoted its practical application by the invention of simplified methods of ascertaining the tidal constants of a port from the observations and of framing tide-tables. In another series of researches the tides of a solid planet of slightly viscous material are investigated, including the consequent secular changes in the motion of the planet and of the tide-generating satellite. He traced from this point of view the past history of the earth and moon, and was led to the now celebrated hypothesis that the latter body originated by fission from its primary when in a molten state.

He has further studied in great detail the classical problem as to the possible figures of equilibrium of a rotating mass of liquid and their respective stabilities, which has engaged in succession the attention of Maclaurin, Jacobi, Kelvin, and Poincaré. The difficult theory of a binary system composed of two liquid masses revolving in relative equilibrium, now known as Roche's problem, has been greatly developed and extended by him. Such investigations have, of course, an important bearing on the theory of the evolution of the earth-moon system already referred to.

The above is a mere summary of the main lines of Sir George Darwin's activity. There are in addition a number of highly important memoirs on more or less cognate subjects. For example, in dealing with the question as to the degree of rigidity of the earth as it now exists, he has treated it from various points of view; he has considered the theory of the long-period tides, and the stresses produced in the interior by the weight of continents and mountain chains. The inferences of Kelvin and Darwin as to a high rigidity have, it is well known, been recently confirmed in a striking manner by the work of Hecker on the lunar disturbance of gravity. It is to be observed in this connection that Darwin's own early attempts (in conjunction with his brother Horace) to measure this lunar effect directly, though not immediately successful, have had a great influence on the subsequent history of the subject, as well as on seismometry.

Mention should also be made of remarkable papers on the history of meteoric swarms, and (in the domain of the more classical astronomy) on periodic orbits.

Sir George Darwin's 'Collected Papers' have now been published in four volumes by the Cambridge University Press. They form a monument of analytical skill and power devoted persistently through a long series of years to the elucidation of a definite series of questions of the highest interest. The difficulties of the tasks to which he has addressed himself are enormous; but, although some of the conclusions only claim as yet to be provisional and speculative, a mass of definite achievement remains which will always rank as one of the most substantial contributions to the study of cosmic evolution.

## ROYAL MEDALS.

The assent of His Majesty the King has been signified to the following awards of the two Royal Medals:—

The Royal Medal on the physical side was assigned to Prof. George Chrystal, of Edinburgh University, on account of his contributions to mathematical and physical science, especially, of late years, to the study of seiches on lakes. Conspicuous in his early years as one of Clerk Maxwell's principal lieutenants, it is to him that we owe the experimental proof of the extreme precision of Ohm's law of electric conduction ('Brit. Assoc. Report,' 1876). His memoir on the differential telephone ('Trans. Roy. Soc. Edin.,' 1880) was a notable early extension of the theory and practice of Maxwell's principles as regards inductances, now become more familiar when power transmission, as well as telephonic intercourse, proceeds by use of alternating currents. His duties as a teacher of mathematics led to the 'Treatise on Algebra,' which, besides being a book of original vein, was the earliest systematic exposition in our language of the more rigorous methods demanded in recent times in algebraic analysis. But this purely mental discipline, and its continuation in various memoirs on abstract mathematics, could not wholly occupy a mind trained originally in the school of physical science. Of late years Prof. Chrystal has been engaged with great success in a most interesting subject of research, in the theory and the observation of the free persisting oscillations of level in lakes, first observed and analysed by Forel on the Lake of Geneva. By this work he has, on the one hand, added a new interest to the scenery and the physical geography of the Highlands, and, on the other hand, has extended the domain of the exact application of the principles of mathematical hydrodynamics.

At the moment when the Council was adjudicating this Medal it was unaware that the illustrious mathematician at Edinburgh was then lying on his death-bed. He had been in failing health for some time, but the latest news was more favourable. The end came, however, before he could learn that a Royal Medal had been assigned to him. In these circumstances it was felt that the award should not be cancelled, but that the Medal should be transmitted to his family as a visible token of the admiration with which the Royal Society regards his life-work. On appealing for the sanction of the Royal donor of the Medal, His Majesty was pleased to approve of our proposal, and to add an expression of his condolence: "The King trusts that you will be so good as to convey to the family the assurance of His Majesty's sincere sympathy in the terrible loss that they have sustained, through which so distinguished a career has been brought to a close." Those who had personal acquaintance with Prof. Chrystal mourn the extinction of a life full of charm and brightness.

The Royal Medal on the biological side has been awarded to William Maddock Bayliss, F.R.S. During the last twenty-five years, the part taken by Dr. Bayliss in the advancement of physiology has, perhaps, been unequalled by any other physiologist in this country. His work has ranged over a wide field. In his earlier papers dealing with the electrical phenomena associated with the excitatory state in glands and contractile tissues, he brought forward results which were, at the time, entirely novel, and have formed the basis of all subsequent investigations. His paper with Starling on the electrical phenomena of the mammalian heart was the first to give the correct form of the normal variation, as confirmed by later investigations with the string galvanometer.

Another subject which has engaged his attention at intervals during the whole of his career has been the question of the innervation of the blood vessels. In conjunction with other workers, he took a prominent part in mapping out the course of the vaso-constrictor fibres through the sympathetic system. More important is his work on vaso-dilator nerves and the part played by them in vascular reflexes. His confirmation of the earlier observations of Stricker, and his proof that the vaso-dilator impulses are carried as "antidromic" impulses in the fibres ordinarily subserving sensation, effected a revolution in our conceptions of nerve conduction, and showed that the law of Bell and Majendie, previously accepted as of universal application, did not express the whole truth, and that, in fact, a nerve fibre is normally the seat of processes which are both centripetal and centrifugal.

A third group of researches is represented by those on the innervation, intrinsic and extrinsic, of the intestines. Up to the appearance of the paper, written by him in conjunction with Starling, on the movements of the small intestine, the whole question was in the utmost confusion. For the first time these observers showed conclusively that the movements of the intestine are under the control of a local nervous system; and, even to the present time, the intestines are the only organs in higher animals which have been shown to be the seat of a local nervous system capable of carrying out co-ordinated reflexes.

A fourth group of papers deals with the mechanism of the pancreatic secretion. These researches, which by themselves would be sufficient to justify the award of the Royal Medal, were also carried out in partnership with his colleague, Prof. Starling. For many years physiologists have assumed the production of internal secretions by different organs which might influence other parts of the body. In these researches on the pancreas the first definite proof was brought forward of the production of a chemical substance in one organ, the duodenum, and its passage by the blood to another organ, the pancreas, as a result of events occurring in the duodenum. The secretion of pancreatic juice on the entry of the acid chyme into the duodenum had been previously regarded as a nervous reflex. Bayliss and Starling showed that it was a chemical reflex, *i.e.*, effected by the production of a specific chemical messenger which travelled by the blood, and not by the stimulation of nerve endings and the passage of impulses through nerves and the central nervous system. They showed, moreover, that this secretin was but a type of a whole group of substances which they designated hormones. The discovery of these hormones, and the precise definition of their nature and of the conditions of their activity, mark an important epoch in the development of our knowledge of the organs of the animal body.

The discovery of secretin afforded for the first time a convenient and easy method of obtaining pancreatic juice in large quantities. The investigation of the properties of pancreatic juice and of the activation of its chief proteolytic ferment by another ferment, enterokinase, secreted by the intestinal mucous membrane, has led Bayliss to a further series of researches on the mode of action of enzymes and on the closely related questions with regard to the nature of colloidal solutions. The value of this work has been universally recognised. The book on the nature of enzyme action in which Bayliss' researches are summarised has already appeared in German, while his most recent work on the osmotic pressure of colloids, as studied in solutions of colloidal dye-stuffs, is a model of the manner in which such investigations should be carried out.

## DAVY MEDAL.

The Davy Medal is this year assigned to Prof. Henry Edward Armstrong, F.R.S., on account of his researches in organic and in general chemistry.

For many years he has been engaged, partly alone and partly in collaboration with many of his students and others, in the investigation of a number of important problems in organic chemistry. His series of memoirs on the terpenes, on the chemical and physical relationships which obtain among the isomerides of the naphthalene and the benzene series, and on physiological chemistry, have established a strong claim for recognition.

In addition to his direct scientific work, he has taken an active part in the discussion and criticism of current theories, and has put forward views on chemical change and on other subjects which have suggested fruitful lines of enquiry. Gifted with a scientific imagination, interested in the work of others, exceptionally well informed as to recent progress not only in chemistry but also in cognate sciences, he has had a stimulating effect on his fellow chemists, and has done much to bring together for their mutual benefit the workers in different fields.

## HUGHES MEDAL.

The Hughes Medal has been assigned to Charles Thomson Rees Wilson, F.R.S., in recognition of the value of his contributions to our knowledge of the nuclei produced in dust-free gases, and of his investigations upon the nature and properties of ions in gases. Following up the well-known work of Aitken on dust nuclei, Mr. Wilson devised a special apparatus for producing a sudden cooling of a gas saturated with water vapour. After completely freeing the gas from dust particles he found that water was condensed on a few nuclei after an expansion of volume greater than 1·25, and that a dense cloud was formed when it exceeded 1·38. This work was in progress at the time of the discovery of X-rays. He immediately tried the effect of passing this radiation through the gas in the expansion chamber, and found that a dense cloud of fine water drops was produced for all expansions greater than 1·25. In this way he showed that the charged ions produced in gases by the X-rays became nuclei for the condensation of water at a definite supersaturation. This investigation was of great importance; for not only did it bring to light a very striking property of the gaseous ions, but it illustrated in a concrete way the process of ionisation in a gas, and the discontinuous nature of electrical charges. By this method each charged ion is rendered visible by becoming a centre of condensation of vapour. In later work he investigated the efficiency of the positive and negative ions respectively as centres of condensation; and he showed that equal numbers of ions were produced by X-rays and by the rays from radioactive substances. The effect of other agencies in producing nuclei in gases was examined in detail. The results of these experiments, which are now classical, were communicated in a series of memoirs published in the 'Philosophical Transactions.'

This condensation property of ions, discovered by Wilson, was utilised by Sir J. J. Thomson to count the number of ions present, and to determine that fundamental electrical unit, the charge carried by an ion in gases. Recently Mr. Wilson has perfected the expansion method to detect the effects of individual $\alpha$- and $\beta$-particles. The path of each $\alpha$- or $\beta$-particle through the gas is marked out by condensation of water upon the ions it produces, and the trails showing the paths of the particles can be photographed. He has also obtained photographs illustrating the distribution of ions due to the passage of X-rays through a gas, which show clearly the trails of the $\beta$-particles liberated from the atoms of matter. These experiments are of the greatest interest and importance, and visualise in a remarkable way the fundamental properties of these radiations.

A further study by this extraordinarily delicate method promises not only to afford a practical means of counting the $\alpha$- and $\beta$-particles in a gas, but also to throw light upon some of the more important and recondite effects produced by the passage of different types of ionising radiation.

Mr. Wilson was one of the first to investigate the so-called natural ionisation of gases; he devised a simple type of electroscope for this purpose, which has come into general use, and he has constructed a tilted electroscope of great sensibility, which is now widely used for measurements of ionisation. He has also directed his attention to atmospheric electricity; he has devised an instrument for measuring accurately the current which passes from the upper atmosphere to the earth, and has determined the value of this current under different conditions.

# *Action of Dissolved Substances upon the Autofermentation of Yeast.*

By Arthur Harden, F.R.S., and Sydney G. Paine.

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(From the Biochemical Department, Lister Institute.)

During experiments upon the permeability of the yeast-cell it was found that, when yeast was immersed in a molar solution of sodium chloride, and allowed to stand at air temperature, the amount of gas produced by autofermentation was considerably greater than that given by a water control.

The production of carbon dioxide by autofermentation of yeast is brought about by the action of at least two enzymes. The reserve material of the cell, for the most part glycogen, is first converted by a glycogenase into a sugar, which in turn is fermented by zymase with the production of alcohol and carbon dioxide. As the rate of autofermentation is considerably less than that produced by the same yeast in presence of excess of sugar, it follows that the rate of autofermentation is controlled by the rate of production of sugar within the cell, in other words, by the rate of action of the glycogenase. An increase in the rate of autofermentation, therefore, indicates greater activity of this enzyme within the cell. In order to investigate the action of solutions of various salts upon the rate of autofermentation of yeast, this was ascertained by measuring the volume of carbon dioxide evolved during successive intervals of time by means of the apparatus described by Harden, Thompson, and Young (1). The yeast employed was prepared from top-yeast as obtained from the brewery by pressing out the wort in a small hand press, it having been demonstrated (2) that practically the whole of the interstitial liquid can be removed in this way. A certain weight of such pressed yeast was carefully weighed into each of the fermentation flasks, and treated with a certain volume of the various liquids under experiment, controls being made with water. The liquids were saturated with carbon dioxide at 25°, the temperature of the water-bath.

## 1. *Effect of Sodium Chloride and other Salts upon the Autofermentation of Yeast.*

When yeast was immersed in molar sodium chloride solution the rates of evolution of gas during the first six successive intervals of 20 minutes were 10·6, 8·4, 6·6, 4·9, 4·8, 4·8 c.c., as against 4·9, 4·2, 3·3, 2·9, 2·8, 2·7 c.c. when the same weight of yeast was immersed in water. In the former case fermentation practically came to an end after six hours, at which time 60 c.c. of gas had been collected as against 31 c.c. from the water control (Table I). In the latter case evolution of gas continued steadily until, after about 60 hours, the volume of gas was identical with that from the sodium chloride experiment.

Table I.—Effect of Sodium Chloride upon the Autofermentation of Yeast.

| Time, in hours. | Cubic centimetres of carbon dioxide evolved by 3 grm. of yeast and 20 c.c. of solution. | |
|---|---|---|
| | Sodium chloride, molar. | Water control. |
| 1 | 25·6 | 12·4 |
| 2 | 40·1 | 20·8 |
| 3 | 49·6 | 24·6 |
| 4 | 55·7 | 27·4 |
| 5 | 58·8 | 29·4 |
| 6 | 59·7 | 31·2 |
| 24 | 65·0 | 49·5 |
| 48 | 67·0 | 61·5 |
| 64 | 67·5 | 67·5 |

This experiment shows that, under the influence of molar sodium chloride, the whole of the fermentable material was decomposed in one-tenth of the time required by the water control.

Experiments were next made in order to determine the optimum concentration of this substance, which would give a maximum rate of autofermentation at the temperature employed.

Table II.—Effect of Varying Concentrations of Sodium Chloride.

| No. | Cubic centimetres of carbon dioxide evolved during the first hour from 4 grm. of yeast + 10 c.c. solution | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water. | NaCl. 0·5 M. | NaCl. 0·6 M. | NaCl. 0·7 M. | NaCl. 0·8 M. | NaCl. 0·9 M. | NaCl. 1·0 M. | NaCl. 1·1 M. | NaCl. 1·2 M. | NaCl. 1·5 M. | NaCl. 1·7 M. | NaCl. 2 M. |
| 42 | 21·7 | — | — | — | — | — | 82·0 | — | 68·5 | 57·7 | 42·5 | 42·5 |
| 47 | — | 35·6 | 37·4 | 41·7 | 43·1 | 44·1 | 42·9 | — | — | — | — | — |
| 48 | — | — | — | 49·0 | 50·6 | 50·1 | 44·7 | 44·2 | 41·9 | — | — | — |
| 48A | 7·5 | 19·5 | — | 24·5 | — | 24·7 | 27·2 | 23·6 | — | 22·2 | — | 12·3 |

These results indicate that the optimum concentration varies slightly for different samples of yeast, but that it approximates to molar; moreover, very slight difference is observable in the effect of concentrations ranging from 0·7 to 1·1 molar.

Experiments made with other salts showed that the phenomenon described for sodium chloride is a general one for all salts, both of inorganic and organic acids. The following salts were all found to give positive results: Chlorides of sodium, potassium, lithium, ammonium, magnesium, calcium, and barium; sulphates of sodium, potassium, ammonium, and magnesium; sodium salts of phosphoric, hexosephosphoric, arsenic, acetic, malic, citric, lactic, pyruvic, and glyceric acids.

With the salts of organic acids, the possibility exists that these may themselves be the source of the carbon dioxide. Neubauer (3) and Neuberg, Hildesheimer, Tir, and Karczag (4, 5, 6) have, in fact, stated that some races of yeast are capable of producing carbon dioxide from salts of lactic, glyceric, pyruvic, oxalacetic, and many other acids. As this phenomenon is accompanied by the disappearance of the acid in question, it can readily be distinguished from that which forms the subject of the present paper.

## 2. *The Nature of the Effect Produced by Salts on the Autofermentation of Yeast.*

It seemed advisable at the outset to ascertain experimentally if the increase in the rate of gas production were actually due to stimulation of the glycogenase, as was to be expected, or of the zymase. The sugar fermentation of 1 grm. of yeast immersed in molar sodium chloride gave only 1·7 c.c. of carbon dioxide per five minutes, as against 4·1 c.c. in the case of a water control. The action of the zymase is therefore inhibited rather than enhanced by this treatment. The increase in the rate of autofermentation would accordingly seem to result from a more efficient working of the glycogenase.

This might be due to one or more of the following causes:—

(1) To some specific action of the salt employed.

(2) To a concentration within the cell by removal of water as a result of plasmolysis.

(3) To removal from the cell of some substance or complex which has an inhibitory or controlling action upon the rate of glycogen fermentation.

(4) To disorganisation of the cell, whereby the factor controlling the access of enzyme to glycogen is in some way modified.

(5) To "hormone" action of the substance on the lines suggested by H. E. and E. F. Armstrong.

(1) *Specific Action.*—In order that a specific action should be exerted, it is essential that the agent should be capable of entering the cell. As regards

this question, in an earlier work* the conclusion was reached that most salts are probably not capable of penetrating beyond the outer layers of the cytoplasm. This would render any specific action upon the enzyme very doubtful. Moreover, it is improbable that so many different salts should exert a similar effect. Further, such action, if exerted in the cell, should also be exhibited in the contents after removal from the cell. The following table shows the result of addition of salt to yeast-juice both in presence and absence of added sugar :—

Table III.—Effect of Sodium Chloride upon Fermentation by Yeast-juice.

| | Cubic centimetres of carbon dioxide evolved by 25 c.c. of yeast-juice in 18 hours. | | | | |
|---|---|---|---|---|---|
| | Control. | + 0·14 grm. NaCl. | + 0·36 grm. NaCl. | + 0·72 grm. NaCl. | + 1·45 grm. NaCl.* |
| Sugar free............ | 35·3 | 28·0 | 18·2 | 8·2 | 2·2 |
| + 1 grm. glucose... | 55·9 | 42·2 | 29·8 | 14·5 | 3·1 |

* Molar concentration.

These numbers prove that the autofermentation is diminished in practically the same proportion as the sugar fermentation, and they afford no evidence of acceleration of the action of the glycogenase.

Very similar results were obtained with zymin.

Table IV.—Effect of Sodium Chloride upon Fermentation by Zymin.

| | Cubic centimetres of carbon dioxide evolved by 5 grm. zymin + 20 c.c. solution in 5 hours. | | | |
|---|---|---|---|---|
| | Water. | M/10 NaCl. | M/4 NaCl. | M/2 NaCl. |
| Sugar free ...................... | 77·2 | 64·0 | 51·7 | 32·4 |
| + 1 grm. glucose ............ | 173·2 | 162·4 | 136·2 | 83·5 |

It follows from these experiments that the direct action of salt upon the enzymes of yeast is that of an inhibitant, and that the acceleration of the autofermentation of yeast by salt cannot be due to a specific effect of the latter. This, however, does not exclude the possibility that certain substances which accelerate the action of yeast-juice and zymin may also exert a specific effect upon the autofermentation.

(2) *Plasmolysis of the Cell.*—It has been demonstrated by Paine that with molar concentration of sodium chloride strong plasmolysis occurs, while decimolar solution produces no such result. The effect of these concentrations upon the autofermentation of yeast is shown in the following table :—

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* Paine, *loc. cit.*

Table V.—Showing Effect of Molar and Decimolar Solutions of Sodium Chloride.

| Time, in hours. | Cubic centimetres of carbon dioxide evolved from 5 grm. yeast and 20 c.c. of solution. | | |
|---|---|---|---|
| | NaCl, molar. | NaCl, decimolar. | Water control. |
| 1 | 37 ·5 | 11 ·2 | 12 ·6 |
| 2 | 57 ·4 | 17 ·4 | 18 ·2 |
| 3 | 66 ·6 | 21 ·4 | 21 ·5 |
| 4 | 70 ·8 | 23 ·9 | 23 ·8 |
| 5 | 71 ·7 | 26 ·1 | 26 ·1 |

It follows that sodium chloride solution is without influence upon the auto-fermentation when the concentration is so low as to produce no plasmolysis of the yeast.

Experiments were, therefore, made to determine the effect of iso-osmotic solutions of various substances, which had all been found to produce plasmolysis in a similar manner to sodium chloride. The osmotic coefficients were taken from the tables given in Pfeffer's 'Physiology of Plants,' and in some cases the freezing points of the solutions were determined. The results are given in the following tables :—

Table VI.—Effect of Iso-osmotic Solutions of Salts.

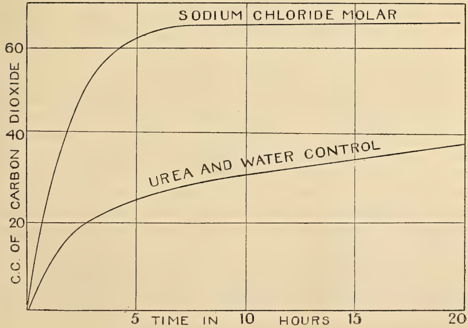
| No. of expt....... | 82.<br>A. | B. | C. | D. | 83.<br>A. | B. | C. | D. |
|---|---|---|---|---|---|---|---|---|
| Details............ | 10 grm. pressed yeast + 20 c.c. of solution. | | | | | | | |
| Substance employed | NaCl | $K_2HPO_4$ | $CaCl_2$ | Water control | NaCl | $K_2SO_4$ | Mannitol | Water control |
| Concentration ... | Molar | 13 grm.<br>100 c.c. | 8·3 grm.<br>100 c.c. | — | ½ molar | 6·5 grm.<br>100 c.c. | 13·5 grm.<br>100 c.c. | — |
| Time, in hours. | Cubic centimetres of carbon dioxide. | | | | Cubic centimetres of carbon dioxide. | | | |
| 0 | — | — | — | — | — | — | — | — |
| 0 ·5 | 18 ·3 | 19 ·6 | 19 ·5 | 10 ·5 | 14 ·6 | 13 ·3 | 12 ·7 | 10 ·5 |
| 1 ·0 | 31 ·8 | 33 ·3 | 33 ·5 | 16 ·4 | 24 ·0 | 22 ·0 | 21 ·0 | 16 ·4 |
| 1 ·5 | 42 ·1 | 42 ·7 | 43 ·3 | 20 ·9 | 30 ·8 | 28 ·0 | 28 ·2 | 20 ·9 |
| 2 ·0 | 49 ·3 | 49 ·3 | 50 ·5 | 24 ·8 | 36 ·0 | 32 ·7 | 31 ·8 | 24 ·8 |
| 2 ·5 | 53 ·6 | 53 ·3 | 54 ·9 | 27 ·8 | 39 ·2 | 35 ·5 | 34 ·6 | 27 ·8 |
| 3 ·0 | 57 ·3 | 56 ·5 | 58 ·5 | 30 ·8 | 41 ·7 | 38 ·0 | 36 ·8 | 30 ·8 |
| 4 ·5 | 62 ·0 | 61 ·2 | 64 ·3 | 36 ·8 | 45 ·7 | 43 ·0 | 41 ·5 | 36 ·8 |

Table VII.—Effect of Solutions Iso-osmotic with 0·5 Molar Potassium Nitrate.

| No. 84 ......... | 10 grm. pressed yeast + 20 c.c. solution. | | | | | |
|---|---|---|---|---|---|---|
| Substance.................. | $KNO_3$ | $MgSO_4$ | $BaCl_2$ | Mannitol. | Glycerol. | Water. |
| Concentration............ | 5 ·05 grm.<br>100 c.c. | 18 ·45 grm.<br>100 c.c. | 9 ·1 grm.<br>100 c.c. | 13 ·65 grm.<br>100 c.c. | 6 ·95 grm.<br>100 c.c. | — |
| Depression of freezing-point | 1 ·46° | 1 ·51° | 1 ·80° | 1 ·57° | 1 ·52° | — |
| Time. | Cubic centimetres of carbon dioxide. | | | | | |
| 12.45 | — | — | — | — | — | — |
| 1.15 | 29 ·5 | 27 ·0 | 30 ·1 | 29 ·2 | 27 ·6 | 19 ·0 |
| 1.45 | 54 ·3 | 50 ·0 | 57 ·8 | 56 ·4 | 50 ·0 | 31 ·6 |
| 2.15 | 75 ·8 | 70 ·0 | 80 ·9 | 78 ·5 | 66 ·6 | 41 ·0 |

These experiments point very strongly to the removal of water from the cell as the essential factor, since it is seen that, when substances which cause plasmolysis are employed, solutions of equal osmotic pressure produce an equal degree of acceleration.

In order to obtain convincing proof of this, it was necessary to find some



substance which would produce no plasmolysis of yeast even in concentrated solution, and to show that it would not cause acceleration. In earlier experiments urea was found to produce no plasmolysis at molar concentration. The determination of the effect of this substance upon the rate of autofermentation was therefore of first importance. In one experiment molar urea was compared with molar sodium chloride and water. The urea was found to be without influence, as shown in the curves (p. 453).

In the following experiment (No. 85) the effects of isotonic solutions of urea, sodium chloride, and potassium nitrate were compared.

Table VIII.—Effect of Urea Solutions.

| No. 85. ......... | 10 grm. yeast + 20 c.c. solution. | | | | |
|---|---|---|---|---|---|
| | NaCl | Urea | $KNO_3$ | Urea | Water |
| Concentration............ | 5·85 grm.<br>100 c.c.<br>= molar | 9·0 grm.<br>100 c.c. | 5·05 grm.<br>100 c.c.<br>= 0·5 molar | 4·5<br>100 c.c. | — |
| Depression of freezing point | — | — | 1·46° | 1·42° | — |
| Time. | Cubic centimetres of carbon dioxide. | | | | |
| 0·0 | — | — | — | — | — |
| 0·5 | 34·0 | 17·0 | 21·3 | 16·5 | 17·0 |
| 1·0 | 65·0 | 29·2 | 46·7 | 29·4 | 29·3 |
| 1·5 | 86·5 | 34·5 | 60·3 | 35·5 | 35·5 |

Urea is thus seen to be without influence upon the rate of autofermentation, although, as shown by the depression of the freezing-point, the solutions of this substance were isotonic with the corresponding salt controls. The fact that plasmolysis of the cells is not produced by urea solutions would seem to indicate that this substance can penetrate freely through the cytoplasm of the yeast cell. An experiment was made to investigate this point, the method described by Paine (2) being employed; 100 grm. of yeast were suspended in 100 grm. of molar urea solution, allowed to stand 20 hours at a temperature approximating to zero, and the distribution of urea determined (Table IX).

Urea is thus seen to penetrate readily into the cells, the factor K representing the coefficient of diffusion being of the same order as that obtained for alcohol, namely, 0·85 to 0·87. Although urea enters the cells it is without influence upon the rate of autofermentation.

Table IX.—Showing Diffusion of Urea into the Yeast-cell.

| | Initial yeast. | Initial liquid. | Final yeast. | Final liquid. | P = grm. urea per 100 grm. water within the cells. | $P_1$ = grm. urea per 100 grm. water outside the cells. | K = $P/P_1$. |
|---|---|---|---|---|---|---|---|
| | grm. | grm. | grm. | grm. | | | |
| Solids other than urea | 32·50 | — | 31·61 | 0·85 | | | |
| Urea ................... | — | 5·94 | 2·50 | 3·45 | | | |
| Water ................... | 67·50 | 94·06 | 72·49 | 89·10 | | | |
| Total weight...... | 100·00 | 100·00 | 106·60 | 93·40 | 3·43 | 3·87 | 0·89 |

*Removal of Water by Partial Drying.*—If the acceleration of the enzymic activity were due simply to concentration within the cell, removal of water by drying would be expected to produce the same result as removal of water by plasmolysis. In order to investigate this 10 grm. of pressed yeast which had been passed through a 3 mm. sieve were placed in a fermentation flask and subjected to a current of air for 20 minutes. This flask and a control were then connected with the gas-measuring apparatus and warmed in the water-bath at 25°. The rate of autofermentation was considerably increased by this simple method of removing water.

Table X.—Effect of Partial Drying by Air.

| Time, in mins. | Cubic centimetres of carbon dioxide given by 10 grm. yeast. | |
|---|---|---|
| | After 20 minutes blow. | Control. |
| 15 | 14·5 | 4·3 |
| 30 | 27·5 | 8·9 |
| 45 | 36·4 | 13·3 |
| 65 | 43·6 | 18·4 |
| 85 | 47·8 | 23·4 |

In another experiment three lots of 10 grm. of pressed yeast were weighed out, of which B and C were dried in a vacuum desiccator for two and four hours respectively, whereby B lost 2 grm. and C 3·2 grm. of water. The rate of autofermentation of these samples was compared against A as control.

Table XI.—Effect of Partial Desiccation *in Vacuo.*

| Time. | Cubic centimetres of carbon dioxide yielded per hour by— | | |
|---|---|---|---|
| | A. 10 grm. yeast. Control. | B. 10 grm. yeast dried 2 hours. | C. 10 grm. yeast dried 4 hours. |
| 1st hour | 36·0 | 46·1 | 55·5 |
| 2nd ,, | 15·3 | 17·2 | 39·3 |
| 3rd ,, | 14·1 | 14·7 | 38·7 |
| 4th ,, | 12·9 | 14·8 | 32·4 |
| 5th ,, | 13·7 | 14·7 | 22·4 |
| 24 hours (total) | 281·6 | 248·3 | 234·9 |

In this experiment a loss of 3·2 grm. of water from 10 grm. of yeast, equal to approximately half the water content of the cells, had the effect of more than doubling the rate of autofermentation.

(3) The possibility of the removal from the cell of some inhibitory or controlling substance during plasmolysis is negatived by these last experiments, wherein the increase of autofermentation was produced under conditions which render such removal impossible unless the substance be a volatile liquid.

(4) The disorganisation of the cell, possibly by the disintegration of a material membrane or network, has been adduced as the cause of some of the effects of anæsthetics on the living cell [Overton (7), Lepeschkin (8), Hans Meyer (9)], and it is not impossible that in certain instances this phenomenon plays some part in the acceleration of the autofermentation of yeast. This possibility is specially present in the case of a substance like toluene, which exerts an anæsthetic effect upon yeast.

The following experiment is typical of many; 10 grm. yeast were mixed with (*a*) 25 c.c. water, (*b*) 25 c.c. water and 5 c.c. toluene, well shaken, and incubated at 25°:—

Table XII.—Effect of Toluene on the Autofermentation of Yeast.

| Time. | Cubic centimetres of carbon dioxide. | | | |
|---|---|---|---|---|
| | *a*. Water. | | *b*. Water + toluene. | |
| | Total. | Rate per 10 mins. | Total. | Rate per 10 mins. |
| 10 mins. | 3 | 3 | 8·5 | 8·5 |
| 20 ,, | 5·3 | 2·3 | 15·5 | 7·0 |
| 30 ,, | 7·6 | 2·3 | 22·7 | 7·2 |
| 40 ,, | 9·4 | 1·6 | 29 | 6·3 |
| 50 ,, | 11·3 | 1·9 | 35·9 | 6·9 |
| 3 hrs. | 21·5 | — | 86·1 | — |

Other instances of the same effect are the following, all of which refer to 10 grm. of yeast:—

Table XIII.—Effect of Toluene.

| Date. | Time, in hours. | Water alone. | Water + toluene. |
|---|---|---|---|
| 3.2.08 | 1 | 4·2 | 29·5 |
| 10.9.09 | 2·5 | 6·3 | 30·4 |
| 17.9.09 | 2 | 6·9 | 34·5 |
| 17.10.07 | 4 | 48·9 | 80·7 |
| 20.10.07 | 5 | 28 | 97·6 |

As in the case of salt solutions the rate slowed down comparatively soon, owing to exhaustion of the fermentable material. The effect is not due to a specific action on the enzymes, since toluene has either no effect or a slight inhibitory effect on the autofermentation of yeast-juice, as is shown by the following result: 25 c.c. of yeast-juice in three hours gave 40·3 c.c. of $CO_2$; in presence of 5 c.c. of toluene the same volume of yeast-juice gave 34 c.c.

It is, however, not impossible that this result may be explicable on the ground of plasmolysis. In spite of the small solubility of toluene in water a considerable degree of plasmolysis is observed when yeast is shaken with water and excess of toluene. Further experiments on this point are in progress.

(5) With regard to the possibility that the foregoing changes may be ultimately due to the action of hormones in the manner suggested by H. E. and E. F. Armstrong (7) no very definite conclusion can be drawn. The action of toluene on yeast undoubtedly presents the closest analogy to that which it exerts on the Aucuba leaf, and it cannot be denied that the various salts employed do penetrate at all events into the outer layers of the yeast cell. Several of the phenomena, however, appeared to be difficult to explain in this way, especially the lack of action of a substance like urea, which penetrates the cell, and the causation of the phenomenon by simple drying. In any case the acceleration caused by salts is accompanied by concentration of the cell contents, so that dilution cannot in these instances be the effective cause, as suggested by Armstrong* for the phenomenon observed by him.

### 3. *Effect of Alcohol on Autofermentation.*

The plasmolysing effect on yeast of solutions of alcohol was found to be practically absent from concentrations up to 10 per cent. (rather more than twice molar), but above this concentration plasmolysis became well marked. The influence of alcohol solutions on the autofermentation of yeast is shown by the following curves, which apply to 10 grm. of yeast:—

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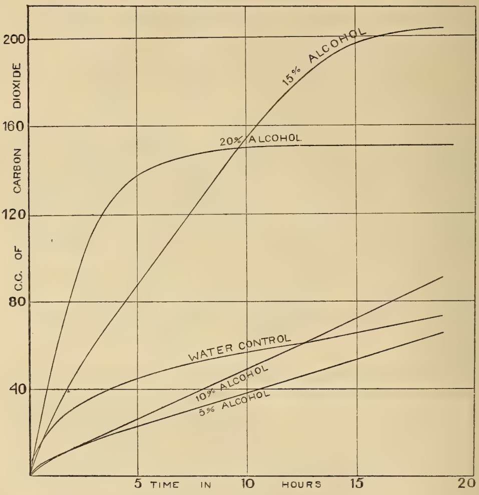
* *Loc. cit.*



Concentrations of alcohol which plasmolyse the cells produce a considerable increase in the rate of autofermentation. With 20 per cent. the action of the enzyme almost came to an end after about seven hours, at which time 147 c.c. of gas had been collected as against 52 c.c. from the water control. The weaker concentrations of alcohol at first produced an inhibitory effect upon the rate. After a short time, however, the rate increased, and then slightly exceeded that of the water control.

Eventually, after eight days, the volume of gas yielded from each, with the exception of that in presence of 20 per cent. alcohol, was practically identical and approximately equal to 200 c.c.

The behaviour of alcohol, therefore, is in accord with that of urea, although the effect is not quite so simple.

### *Summary.*

1. All dissolved substances which plasmolyse the yeast-cell also cause a large increase in the rate of autofermentation.

2. Substances such as urea, which even in concentrated solution do not produce plasmolysis, have no accelerating effect.

3. Toluene produces a similar effect to concentrated salt solutions.

4. The effect produced by salts is probably a direct result of the concentration of the cell contents due to plasmolysis, but in the case of toluene it is possible that some other factor (such as disorganisation of the cell, or hormone action) is concerned.

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# *Further Experiments upon the Blood Volume of Mammals and its Relation to the Surface Area of the Body.*

By Georges Dreyer, M.A., M.D., Professor of Pathology in the University of Oxford, and William Ray, M.B., B.Sc., Philip Walker Student in the University of Oxford.

(Communicated by Prof. Francis Gotch, F.R.S. Received October 24,—Read December 7, 1911.)

(From the Department of Pathology, University of Oxford.)

## (Abstract.)

In a previous paper* dealing with the blood volume of mammals kept in captivity, such as tame rabbits, guinea-pigs, and mice, we have shown that the blood volume is a function of the surface, and can be expressed by the formula $B = W^{\frac{2}{3}}/k$, where B is the blood volume in cubic centimetres, W the weight of the animal in grammes, and $k$ a constant calculated from the experiments, and varying for each species of animal.

In the present paper we have extended our observations upon the blood volume to animals living a natural life in the wild condition, such as hares, wild rabbits, and wild rats. The technique employed was exactly the same as in our previous paper.

The results obtained are in complete accord with our previous experiments, in that the blood volume of each of the wild animals in question is a function of the surface. The constant, determined from the experiments, and from which the blood volume of these animals can be calculated according to the formula $B = W^{\frac{2}{3}}/k$, is for—

| | | | | | |
|---|---|---|---|---|---|
| Hare............ | 0·94 | New series. | Tame rabbit... | 1·58 | Old series. |
| Wild rabbit... | 2·04 | | Guinea-pig ... | 3·30 | |
| Wild rat ...... | 3·05 | | Mouse ......... | 6·70 | |

For all experimental work where the blood volume is concerned, it is necessary to know, not only what the absolute blood volume is, but also, what is equally important, the magnitude of the deviations from the average which may be met with in normal and healthy individuals, since otherwise it is impossible to decide whether the blood volume found by experiment is to be considered normal or abnormal.

Calculating from the total number of *our* experiments by the method of least squares, the mean deviation is found to be about 6 per cent. This

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* Dreyer, Georges, and Ray, William, 'Phil. Trans.,' 1910, B, vol. 201, pp. 133—160.

indicates that if an animal is found by a reliable experimental method to contain 12 per cent. more or less blood than is deduced by calculation from the surface, the average constant of the species being used, it is *probable* that the blood volume of the animal is abnormal, whilst, if it is 20 per cent. smaller or larger, it is *almost certain* that the blood volume is abnormally small or large.

It may be pointed out, however, that if the blood volume were expressed as a percentage of the weight, it would only be possible to say with the same degree of certainty that the blood volume of an animal was abnormal when it differed by at least 40 per cent. from the calculated figure.

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# *The Origin and Destiny of Cholesterol in the Animal Organism.* Part VIII.—*On the Cholesterol Content of the Liver of Rabbits under Various Diets and during Inanition.*

By G. W. Ellis and J. A. Gardner.

(Communicated by Dr. A. D. Waller, F.R.S. Received November 8,—Read December 7, 1911.)

(From the Physiological Laboratory of the University of London, South Kensington.)

In Parts V* and VII† of this series of papers evidence was brought forward to show that when cholesterol, free and in the form of esters, is given with the food of rabbits, some is absorbed and finds its way into the blood stream, and that an increase of both free cholesterol and cholesterol esters takes place in the blood.

This result affords support to the working hypothesis with regard to the origin and destiny of cholesterol in the animal organism, which we were led to formulate in an earlier paper,‡ viz., that cholesterol is a constituent constantly present in all cells, and when these cells are broken down in the life process the cholesterol is not excreted as a waste product but is utilised in the formation of new cells. A function of the liver is to break down dead cells, *e.g.*, blood corpuscles, and eliminate their cholesterol in the bile. After the bile has been poured into the intestine in the processes of digestion, the cholesterol is re-absorbed, possibly in the form of esters, along with the bile salts and is carried in the blood stream to the various centres and tissues for re-incorporation into the constitution of new cells.

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 230—247.

† 'Roy. Soc. Proc.,' 1910, B, vol. 82, pp. 559—568; see also Pribram, 'Biochem. Zeit.,' 1906, vol. 1, p. 413.

‡ 'Roy. Soc. Proc.,' 1908, B, vol. 81, pp. 110—128.

It seemed to us that valuable data for the elucidation of the cholesterol problem might be obtained by a careful study of the cholesterol and cholesterol-ester content of the various organs and tissues of the body, in the case of rabbits fed on diets containing varying amounts of cholesterol, and also of rabbits kept in a state of inanition. In this paper we give an account of our experiments on the cholesterol content of the livers of such rabbits.

### *Method of Estimating the Cholesterol.*

The animals after anæsthetisation were bled as completely as possible. The livers were then taken out and weighed. The material was then finely ground with sand and plaster of Paris. The ground mass was then allowed to set, after which it was finely powdered and extracted in Soxhlet's apparatus with ether for from two to three weeks. The ethereal extracts were made up to a known volume with ether and carefully divided into two equal parts. One part was evaporated to dryness, dissolved in alcohol, and the free cholesterol directly estimated. The other half of the ethereal solution was saponified with sodium ethylate. After separating the soaps, the total cholesterol in the ethereal solution was estimated. The ester cholesterol was determined by difference. The cholesterol was estimated by the digitonin method of Windaus,* using the modified procedure fully described in Part VII of this series of papers.†

### *Cholesterol Content of the Livers of Rabbits fed on Green Food only.*

For this purpose a diet of cabbage leaf and cabbage stalk was selected, as these substances appear to form an efficient diet. Further, cabbage is rich in vegetable sterols which are largely passed unchanged in the fæces. This formed a convenient means of obtaining these sterols, which were required for another purpose, from the plant in quantity.

*Experiment I.*—A strong healthy rabbit (A) was fed on cabbage leaf from December 28, 1910, to February 1, 1911. It took 1·5 lbs. of leaf per day. Twice during the experiment the cabbage leaf was mixed with some extracted bran to prevent the fæces getting too moist, as they were required for another purpose. The rabbit maintained a practically constant weight during the feeding period, as the following weighings show: December 28, 2·7 kgrm.; December 31, 2·5 kgrm.; January 1, 2·6 kgrm.; January 7, 2·45 kgrm.; January 11, 2·5 kgrm.; January 20, 2·5 kgrm.; January 23, 2·5 kgrm.; January 30, 2·5 kgrm.; February 1, 2·6 kgrm. When the animal was killed the stomach was full. The liver weighed 115·45 grm., and was unusually large for an animal of this weight, being 4·44 per cent. of the body weight. The total cholesterol, free and combined, was found to be 0·2425 grm., and the free cholesterol 0·2294 grm. The ester cholesterol was therefore 0·0131 grm.

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* Windaus, "Über die Entziftung der Saponin durch Cholesterin," 'Ber. d. Deutsch. Chem. Ges.,' 1909, vol. 42, pt. 1, p. 238, and 'Zeit. für physiol. Chem.,' 1910, vol. 65, p. 110.

† 'Roy. Soc. Proc.,' 1910, B, vol. 82, pp. 559—568.

*Experiment II.*—This rabbit (B) was fed on cabbage stalks from December 28, 1910, to February 16, 1911. It consumed 1·5 lbs. per day, and on two occasions during the feeding period it was also given some ether-extracted bran. The weight of the animal remained constant, the following being the weights during the feeding: December 28, 2·4 kgrm.; December 31, 2·35 kgrm.; January 3, 2·5 kgrm.; January 7, 2·45 kgrm.; January 11, 2·5 kgrm.; January 20, 2·5 kgrm.; January 23, 2·45 kgrm.; January 30, 2·3 kgrm.; February 6, 2·4 kgrm.; February 9, 2·4 kgrm.; February 11, 2·4 kgrm.; February 13, 2·34 kgrm.; February 16, 2·4 kgrm. The animal was killed on February 16 and the stomach was full. The liver weighed 82·91 grm., *i.e.*, 3·45 per cent. of the body weight. The total cholesterol, free and combined, was 0·1932 grm., and the free cholesterol 0·1228 grm. The ester cholesterol was then 0·0704 grm.

### *Cholesterol Content of the Livers of Rabbits fed on Bran which has been thoroughly extracted with Ether to remove all Fat and Phytosterols.*

This diet was selected, as previous experiments had shown that rabbits can be kept at constant weight for many days together on this food. As the food was sterol-free, the influence of any absorption of vegetable sterols on the cholesterol content of the livers was eliminated.

*Experiment III.*—A healthy buck (C), weighing 2·3 kgrm., was fed on as much bran as it would consume from April 1 to April 8, 1910. The weights taken occasionally during the diet period were 2·3, 2·3, 2·2, 2·15, 2·2, 2·2, 2·2 kgrm. The liver weighed 62·2 grm., *i.e.*, 2·83 per cent. of the body weight. The total cholesterol, free and combined, was 0·1596 grm., and the free cholesterol 0·124 grm. The ester cholesterol was, therefore, 0·0356 grm.

*Experiment IV.*—This was a large doe rabbit (D), weighing 3·1 kgrm. It was fed from March 27 to April 11, 1911, and consumed during this period 1160 grm. of the bran. The weights of the animal taken occasionally were 3·1, 3·2, 3·1, 2·9, 2·9, 2·9, 3·1, 3·1 kgrm. The liver weighed 69·63 grm., or 2·25 per cent. of the body weight. The total cholesterol, free and combined, was 0·232 grm., free cholesterol 0·1527 grm., and ester cholesterol 0·0793 grm.

### *Cholesterol Content of the Liver of Rabbits fed on Extracted Bran to which Free Cholesterol had been added.*

It has already been proved that when cholesterol is given with the food of rabbits, a portion only is excreted in the fæces, the remainder being absorbed in the intestine, giving rise to a well-marked increase in the cholesterol content of the blood. On the hypothesis mentioned at the beginning of the paper we should expect to find in such animals an increase in the cholesterol content of the liver.

*Experiment V.*—A healthy rabbit (E), weighing 2·8 kgrm., was fed from March 27 to April 14, 1911, on extracted bran to which cholesterol was added. It consumed during the period 1480 grm. of extracted bran, and 4·8 grm. of cholesterol. The cholesterol was given daily in 0·25 grm. portions mixed with a small quantity of the moistened bran, and care was taken that the animal ate the whole. The weights of the rabbit taken occasionally were 2·8, 2·8, 2·8, 2·7, 2·7, 2·7, 2·8, 2·8, 2·6, 2·6 kgrm. It thus lost during the whole period 0·2 kgrm.

The weight of the liver was 72·25 grm., *i.e.*, 2·77 per cent. of body weight. The liver contained 0·3315 grm. of cholesterol, and there was no ester present.

*Experiment VI.*—In this experiment a rabbit (F) was fed with as much extracted bran as it would eat. On the 1st, 2nd, 4th, and 5th days it received 0·25 grm. of cholesterol mixed with a little moist bran, and on the 6th, 7th, and 8th days 0·5 grm. It thus had 2·5 grm. during the period. The weights of the rabbit were as follows: 2·8, 2·7, 2·65, 2·6, 2·6, 2·65, and 2·7 kgrm. The liver weighed 74·7 grm., *i.e.*, 2·76 per cent. of body weight. It contained 0·341 grm. of free and combined cholesterol, 0·2144 of free cholesterol, the ester cholesterol thus being 0·1266.

### *Experiments in which Rabbits were fed on Extracted Bran, but the Cholesterol, instead of being given by the Mouth, was injected in Olive Oil Solution into the Peritoneal Cavity.*

In order to ascertain whether cholesterin absorbed from other parts of the body than the intestine would be carried to the liver and cause an increase in the cholesterol content of that organ, two rabbits were fed on extracted bran. In one, the control, pure olive oil was injected into the peritoneal cavity, and in the other a solution of cholesterol in olive oil.

After recovering from the operations and feeding for some days the animals were killed and their livers analysed.

*Experiment VII.*—As a control a rabbit (G), weighing 3·4 kgrm., was anæsthetised with ether on July 13, and 10 c.c. of sterilised olive oil injected into the peritoneal cavity. The animal did not eat well until July 18, when its weight was 2·8 kgrm. After this date it took its food (extracted bran) readily, and its weight remained quite constant until July 28. On the following day another 10 c.c. of olive oil was injected as before. It was killed on August 6, when its weight was 2·4 kgrm. The liver, which was normal in appearance, weighed 51·27 grm., *i.e.*, 2·05 per cent. of body weight. Some oil still remained unabsorbed in the cavity. The weight of free and combined cholesterol in the liver was found to be 0·1698 grm., the free cholesterol 0·1418 grm., and the ester cholesterol, by difference, 0·028 grm.

The fæces of the animal were collected during the whole experiment, and after drying weighed 364 grm. They were extracted with ether, and the fats in the ethereal solution saponified with sodium ethylate. After separating the soaps and washing, the ethereal solution was evaporated to dryness. The oily residue obtained was taken up in alcohol and precipitated with digitonin. The precipitate was thoroughly washed with ether and then with water, and after drying weighed 0·254 grm. This precipitate was decomposed by heating in xylene vapour, according to the method of Windaus for recovering the cholesterol from its digitonin compound.

After evaporating the xylene and crystallising from alcohol, crystals were obtained which under the microscope had the form of typical cholesterol crystals. The fæces therefore contained 0·0617 grm. of cholesterol, an output of 0·0028 grm. per day.

*Experiment VIII.*—A vigorous rabbit (H), weighing 3·7 kgrm., was anæsthetised with ether, and 10 c.c. of olive oil, containing 0·5 grm. of cholesterol in solution, injected into the peritoneal cavity on July 13. As in case of rabbit (G) it took very little food (extracted bran) until July 16, when its weight was 3 kgrm. The weight remained fairly constant until July 29, when it weighed 2·8 kgrm. Another 10 c.c. of oil containing 0·5 grm. of cholesterol was again injected. The animal was killed on August 6, when its weight was 2·8 kgrm. Some of the oil was still unabsorbed. The liver, normal in appearance, weighed 59·01 grm., or 2·1 per cent. of the body weight. It contained 0·3485 grm. of free cholesterol and no ester cholesterol. During the experiment it passed 669 grm. of fæces (dry). This was treated as in the control experiment, and 0·695 grm. of digitonin compound was obtained, corresponding to 0·1689 grm. cholesterol, an output of 0·0073 grm. per day.

### *Cholesterol Content of the Livers of Rabbits during Inanition.*

If the hypothesis put forward at the beginning of this paper is correct, we should expect that, during inanition, when an animal is living on its own tissues and the ordinary processes of digestion are in abeyance, an accumulation of cholesterol would take place in the liver. In order to test this, two rabbits, which had been long in stock and well fed, were selected. One was a fat animal, and the other a thin one, which, though well fed, showed little tendency to lay on fat.

*Experiment IX.*—This animal (I), at the beginning of the experiment, was fat, and weighed 3 kgrm. It was fed for three days on extracted bran, after which it was kept without food from October 28 to November 3, 1910, but was allowed water *ad lib.* It steadily decreased in weight: 2·9, 2·8, 2·65, 2·6, 2·5, 2·45 kgrm., and at the end of the period was apparently in good health. It appeared to suffer no inconvenience. It passed no fæces during the inanition period. After it had been killed, it was found that there was still some fat round the kidney and in other parts. The stomach and intestines contained a dark semi-fluid material, and the stomach was full of wind. Some fæces were found in the rectum. The gall bladder was distended. The loss in weight was 18 per cent. The liver was normal in appearance and weighed 43·01 grm., *i.e.*, 1·75 per cent. of the body weight. The total cholesterol, free and combined, was 0·3406 grm., and the free cholesterol 0·1831 grm. The ester cholesterol, by difference, was thus 0·1575 grm.

*Experiment X.*—This animal (J) was vigorous but thin, and at the commencement of the experiment weighed 1·9 kgrm. It was fed for three days on extracted bran, after which it was kept without food from November 11 to 17, 1910, but allowed plenty of water. It lost weight steadily, the weights being 1·8, 1·7, 1·6, 1·55, 1·45, 1·4, a percentage loss of 26·2. The animal suffered no obvious inconvenience during the fast. It was killed on November 17. The stomach contained a dark semi-fluid matter, and there were some fæces in the rectum. No fat was noticed round the organs. The animal passed no fæces during inanition. Unfortunately, the dark matter in the stomach and intestines was not analysed. The liver was normal in appearance and weighed 32·87 grm., *i.e.*, 2·35 per cent. of the body weight. The total cholesterol, free and combined, was 0·1234 grm., the free cholesterol 0·1123, and the ester cholesterol, by difference, 0·0111 grm.

*Cholesterol Content of the Livers of Newly-born Rabbits.*

*Experiment XI.*—Five newly-born animals were taken, the mother having been fed on an ordinary mixed diet of bran, oats, and green stuff.

The livers of the five animals weighed 17·12 grm. They were found to contain 0·0369 grm. of cholesterol, free and combined, 0·0317 grm. of free cholesterol. The ester cholesterol, by difference, was 0·0052 grm.

The results of the above 11 experiments are gathered together in the following table (p. 468).

*Discussion of Results.*

On comparing the figures in the following table it will be seen that in Experiments III, IV, and VII, on animals fed on extracted bran alone, the total free and combined cholesterol per kilogramme of body weight is remarkably constant. This figure may be taken as representing the normal cholesterol content of the liver under conditions in which the body weight is kept constant, but no cholesterol or phytosterol is absorbed with the food. On comparing these figures with those in Experiments I and II, in which the animals had been fed for a very long period on green food containing phytosterol, a small increase is noticed, indicating that some phytosterol was absorbed from the food and appeared in the liver in the form of cholesterol. It would of course require a much larger number of experimental data to be certain on the point, but the result is in agreement with the observations on blood published in Part VII of the series, in which a similar increase in the cholesterol content of the blood of rabbits fed on extracted bran *plus* phytosterol compared with that of similar animals fed on extracted bran alone was observed. If we consider the percentage contents of the livers themselves the increase is not observed. It will be noticed, however, that the livers of the two animals fed on green cabbage are extraordinarily large compared with those of the other animals of about the same weight. Whether this is accidental or brought about by the nature of the food we are unable to say, though the animals, as far as general and *post-mortem* appearances were concerned, seemed to have been in good health.

In the case of the animals E and F, fed on extracted bran to which an excess of cholesterol had been added, or H, in which the cholesterol was injected into the peritoneal cavity, a marked increase in the total cholesterol of the liver is noticeable, no matter whether the actual cholesterol found, or the percentage in the liver, or the weight per kilogramme of body weight is considered. This increase is much too large, we consider, to be due to chance.

In Experiments IX and X, on animals kept in a state of inanition and

| Number of experiment and letter of rabbit. | Food of rabbit. | Weight of animal when killed, in kgrm. | Weight of liver, in grammes. | Weight of total cholesterol free and combined, in grammes. | Weight of free cholesterol, in grammes. | Weight of ester cholesterol, in grammes. | Weight of cholesterol per cent. of liver. | | | Weight of liver cholesterol per kgrm. of body weight. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total, free and combined. | Free cholesterol. | Ester cholesterol. | Total, free and combined. | Free. | Ester. |
| I, A | Cabbage leaf......... | 2·60 | 115·45 | 0·2425 | 0·2294 | 0·0131 | 0·211 | 0·199 | 0·012 | **0·093** | 0·088 | 0·005 |
| II, B | Cabbage stalk ...... | 2·40 | 82·91 | 0·1932 | 0·1228 | 0·0704 | 0·233 | 0·148 | 0·085 | **0·081** | 0·051 | 0·030 |
| III, C | Extracted bran...... | 2·20 | 62·20 | 0·1596 | 0·1240 | 0·0356 | 0·257 | 0·199 | 0·057 | **0·073** | 0·056 | 0·017 |
| IV, D | Extracted bran...... | 3·10 | 69·63 | 0·2320 | 0·1527 | 0·0793 | 0·333 | 0·219 | 0·114 | **0·075** | 0·045 | 0·030 |
| VII, G | Extracted bran (oil injected into peritoneal cavity) | 2·40 | 51·27 | 0·1698 | 0·1418 | 0·0280 | 0·331 | 0·277 | 0·054 | **0·071** | 0·059 | 0·012 |
| V, E | Extracted bran and cholesterol | 2·60 | 72·25 | 0·3315 | 0·3315 | Nil | 0·459 | 0·459 | Nil | **0·128** | 0·128 | Nil |
| VI, F | Extracted bran and cholesterol | 2·70 | 74·70 | 0·3410 | 0·2144 | 0·1266 | 0·457 | 0·287 | 0·170 | **0·126** | 0·079 | 0·047 |
| VIII, H | Extracted bran (cholesterol injected into peritoneal cavity) | 2·80 | 59·01 | 0·3485 | 0·3485 | Nil | 0·591 | 0·591 | Nil | **0·125** | 0·125 | Nil |
| IX, I | No food (water allowed) | 2·45 | 43·01 | 0·3406 | 0·1831 | 0·1575 | 0·792 | 0·426 | 0·366 | **0·139** | 0·075 | 0·064 |
| X, J | No food (water allowed) | 1·40 | 32·87 | 0·1234 | 0·1123 | 0·0111 | 0·376 | 0·286 | 0·090 | **0·088** | 0·080 | 0·008 |
| XI | Five newly-born rabbits (unfed) | — | 17·12 | 0·0369 | 0·0317 | 0·0052 | 0·216 | 0·185 | 0·031 | — | — | — |

living on their own tissues, we find, as was expected, a similar storing up of the cholesterol in the liver. This is very marked in the case of the fat rabbit in Experiment IX, which probably used up the fat directly, and less marked in the case of the lean rabbit in Experiment X, which made a greater demand on its tissues. This result, we think, was also to be expected. In Experiment XI the percentage total cholesterol content of the liver of newly-born rabbits is of the same order of magnitude as that of adult rabbits. What factors govern the relative proportions of free cholesterol and cholesterol esters the experiments do not indicate.

These results we submit afford striking evidence in support of the hypothesis advanced at the beginning of this paper.

### *Addendum on the Examination of the Unsaponifiable Matter in the Fæces of Rabbits fed on Ether-extracted Bran.*

In former investigations on the subject we never succeeded in crystallising cholesterol from the fæces of rabbits fed on extracted bran, but, as in Experiment VIII we succeeded in isolating cholesterol by the digitonin method from the fæces of rabbit G, which had a diet of extracted bran, but into the peritoneal cavity of which olive oil had been injected, it became necessary to examine more clearly the fæces of a normal animal fed in the same way. For this purpose a rabbit was fed for 15 days on extracted bran and the fæces collected. They weighed, after drying, 295 grm., and yielded, after treatment in the manner described, 0·6445 grm. of unsaponifiable oily matter, soluble in alcohol. This residue was dissolved in alcohol and mixed with as much of an alcoholic solution of digitonin as would have completely precipitated the residue had it consisted of pure cholesterol. This was allowed to evaporate to dryness spontaneously, and was obtained free from unchanged oil by means of ether. This oil weighed 0·3076 grm., and did not give any sterol colour reaction in chloroform solution with acetic anhydride and sulphuric acid. The digitonin precipitate, after washing repeatedly with hot water, weighed 1·2175 grm., but it was not free from digitonin, and was yellowish in colour. It was then finely powdered and washed with about 200 c.c. of ether until colourless. The ether dissolved about 0·25 grm. of solid matter. This solid was insoluble in petroleum ether, but soluble in benzene, and on testing with acetic anhydride and sulphuric acid only gave the sterol colours in a slight and indefinite manner. The 0·99 grm. of digitonin compound was then heated in xylene vapour until completely decomposed, and the clear xylene solution on evaporation gave a yellow, oily solid. This was only partly soluble in ether, leaving a white, insoluble powder, which did not give any sterol reaction. The ethereal solution was evaporated and the solid recrystallised from 95-per-cent. alcohol. The crystalline matter which separated was impure, and, even after recrystallisation, we could recognise under the microscope no crystals which could be definitely described as cholesterol. The whole was then converted into benzoate by the action of benzoyl chloride in pyridin solution; 0·068 grm. of a benzoate was obtained, which, after repeated recrystallisation from alcohol, was still slightly yellow in colour. It melted at 142° C. to a clear brown liquid, which, on cooling, gave a brilliant *green* play of colours at the moment of solidification, gradually changing to brown. This behaviour was quite different from cholesterol benzoate, which melts at 145° to a turbid liquid, only becoming clear at about 180°, and, on cooling, gives a play of purple and blue colours of quite characteristic appearance. Under the microscope the crystalline matter was indefinite in appearance and one could find none of the characteristic square envelopes of cholesterol benzoate.

Had the 0·97 grm. of insoluble digitonin compound consisted entirely of cholesterol digitonide, it would have corresponded to 0·24 grm. of cholesterol, an output of only 0·016 grm. per day.

In order to determine satisfactorily the nature of the unsaponifiable residue, it will be necessary to prepare it in large quantity, and this we must reserve for a further investigation.

We take this opportunity of thanking the Government Grant Committee of the Royal Society for help in carrying out this work.

# *Herbage Studies.* I.—Lotus corniculatus, *a Cyanophoric Plant.*

By Henry E. Armstrong, F.R.S., E. Frankland Armstrong and
Edward Horton.

(Received and read November 23, 1911.)

Hitherto little attention has been paid to the individual plants which constitute the herbage of pasture lands and no serious attempt has been made to appraise their quality; this is the more surprising, as it is well known that certain pastures are of special value as grazing lands and that the food value of herbage often differs to an extraordinary extent in different districts and even in neighbouring fields—so much so that it is impossible to fatten cattle on many, if not on the majority, of pastures; moreover, there are marked differences depending on seasonal conditions. It is clear that such differences may be due both to variation in the botanical composition of the herbage and to variation in the composition of individual plants induced by variation in soil and in climatic conditions; at present, however, it is impossible even to hazard an opinion as to the manner in which these and doubtless other factors are operative.

Our present difficulty arises from the lack of methods of appraising quality: we are no longer satisfied with determinations of dry matter, digestible matter and albuminoid nitrogen, now that we realise that *quality* as much as *quantity* is of importance—that in the case of cattle, as in our own case, a mixed and varied diet is required and that what may be termed the condimental constituents of food are, perhaps, at least equal in importance to those which serve exclusively as building materials or as a source of energy. An increasing weight of evidence appears to be in favour of the view that the vital processes in plants as well as in animals are controlled in greater or less degree by substances of the class we have proposed to designate collectively as Hormones. There can be little doubt, in fact, that it will be necessary to take many factors into account in appraising the value of foods—far more, indeed, than it has been customary to consider hitherto.

It is not at all improbable that the glucosides present in plants in small quantity are in some cases of definite *condimental* value. A case in point is that of linseed. Owing to the presence of the glucoside linamarin (phaseolunatin) in the unripe seed, a small quantity of hydrogen cyanide is usually to be found in linseed cake. It is well known that this cake has qualities which make it superior to all other seed cakes as a food in bringing cattle into condition; it may well be that it owes its superiority to this small amount of hydrogen cyanide and perhaps also to the acetone that accompanies the cyanide. Arrangements have been made which it is hoped will permit of this problem being solved.

We have been fortunate in having our attention attracted to a plant the study of which promises to be of interest not only from the point of view above set forth but also for other reasons which will be apparent when our account is considered. In the course of our search for enzymes of the emulsin type* we have examined a large number of Leguminosæ and were led, early in the summer of last year, to discover in *Lotus corniculatus* (Bird's-foot trefoil) a plant in which such an enzyme is associated with a cyanophoric glucoside. We may mention that another reason which led us to select this plant and test it for hydrogen cyanide was the fact that Dunstan and Henry had discovered this substance in *Lotus arabicus*—a plant growing on the banks of the Nile—and that hydrogen cyanide had also been found in *Lotus australis.*

The first specimen tested was picked on the Thames, near Wargrave, in June, 1910. It was found to contain hydrogen cyanide when tested by Guignard's alkaline picrate paper: a slip of the yellow paper, enclosed in a small tube with two or three grammes of the plant and a drop or two of chloroform, soon darkened in colour and ultimately became brick-red. This specimen of *Lotus corniculatus* was also found to contain an enzyme or enzymes which acted readily both on linamarin and on prunasin† though but slightly on amygdalin.

Of several specimens obtained from the Reading district soon after the first was picked, only one or two contained hydrogen cyanide; moreover, the cyanide could not be detected in a number of specimens picked in July in the Harpenden district and also near Flitwick (Beds.).

During the early part of August search was made for the plant all over the Swanage district, in Dorsetshire. It was found growing on London clay, on chalk, on Purbeck and Portland limestone and on Kimmeridge clay but only in one or two cases was hydrogen cyanide detected; no difference was apparent between the plants from the various soils.

In the latter part of August we met with the plant in Switzerland, in the Saas Valley; again no evidence of the presence of hydrogen cyanide was obtainable.

In September and October we obtained a second set of specimens from the Harpenden and Flitwick districts; these also were tested without cyanide being discovered.

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* Compare 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 349.

† We propose to use this name for the glucoside prepared from amygdalin—*amygdo-* or *mandelo-nitrile-glucoside*—sometimes spoken of by us in earlier communications as Fischer's glucoside.

One specially interesting result of the work done at this time may be mentioned here. The form of *Lotus corniculatus* which some botanists regard as a mere variety and others as a distinct species, *Lotus major* or *Lotus uliginosus,* which grows, as a rule, in damp situations, was found to be free not only from hydrogen cyanide but also from the correlated enzyme. This variety is distinguished by its rank growth and coarse tubular stem.

The conclusion we arrived at last year was, therefore, that *Lotus corniculatus* occasionally contained a cyanophoric glucoside and corresponding enzyme but we had no reason to connect the presence of the glucoside with any particular conditions either of soil or of climate.

This year the first specimen of Lotus we examined was sent to us from Portrush, in North-East Ireland, by Dr. J. Vargas Eyre, who early in May found a dwarf form of the plant growing there in profusion on the sand dunes. This proved to be rich in hydrogen cyanide and also contained an active enzyme. Dr. Eyre obtained other specimens in Ireland during May; all of these were cyanophoric.

At Whitsuntide, however, one of us tested a considerable number of specimens in Ayrshire, in the Barrhill district, always without finding any trace of cyanide; but on going out to the coast at Ballantrae again a stunted form of *Lotus corniculatus* was found growing in profusion on the beach just above high-water mark and this plant contained both cyanide and enzyme but other specimens obtained on the same day from the hillside overlooking the beach and only a short distance from it were free from cyanide.

Having found cyanide only in the two stunted forms of the plant grown on sea-sand at the coast, we were led to think that the occurrence of the cyanophoric glucoside might possibly be favoured by "starvation conditions," especially as the conditions during the previous year and in the Ayrshire district early in the present season had been such as to favour luxuriant growth.

During the present summer specimens have been procured from many localities; the result of testing these has been to show that whereas last year cyanide was rarely present, this year it has rarely been absent. We have never failed to detect it in plants from the neighbourhood of Reading, grown under all sorts of conditions, excepting always the form definitely recognisable as *Lotus corniculatus* var. *major* (*uliginosus*); wherever we have obtained this form, it has always proved to be free from cyanide and we have also confirmed our observation made last year that this variety is free from the enzyme which occurs in the cyanophoric form.

Plants growing this year under a great variety of manurial conditions on the experimental grass plots at Rothamsted have always contained cyanide;

last year we never succeeded in detecting it in plants growing on these plots. But it is very noteworthy that on several occasions this year we found patches of the plant in the Harpenden district growing near to one another which were markedly different, the one being rich in cyanide the other containing little if any. Thus of five separate patches found on July 1 in a field of lucerne, only three contained an appreciable amount of cyanide; of two patches growing close together at the edge of a wheat field only one contained cyanide; a case similar to this latter was met with at Redbourn, a few miles from Harpenden.

We had a like experience with plants from Yorkshire. Mr. Harold Wager was good enough to send us seven specimens collected early in July near Threshfield, in Yorkshire, at spots which appeared to afford somewhat different conditions; five of these were rich in cyanide, whilst two contained but traces.

Plants collected in various places in the Isle of Wight in August were all very rich in cyanide. It was also found in plants growing in the Swanage district in places where none could be detected in the specimens collected last year.

Plants have been raised by one of us, at Lewisham near London, from seed gathered last year at Kimmeridge from plants (growing on the cliff face in disintegrated Kimmeridge shale) which did not then contain cyanide. From an early stage onwards up to the present date (November 20, 1911), these have always contained cyanophoric glucoside and the attendant enzyme. We regard this as a result of special importance.

Plants have also been raised from seed obtained early in the year from Messrs. Vilmorin, of Paris, at Lewisham, at University College, Reading, and on four of the barley plots at Rothamsted—1A, 2A, 3A and 4A; these have always been rich in cyanide. Plants raised at Lewisham and Reading from seed purchased from Messrs. Vilmorin as that of *Lotus major* var. *villosus* have shown no trace of cyanide and have also been free from enzyme.

Plants obtained at West Horsham in July, at Margate in September and at half a dozen different localities in the Sidmouth (Devonshire) district, also in September, were all cyanophoric.

One other experience remains to be related with reference to the British Isles. Early in September, on visiting St. Andrews at the time of the celebration of the 500th Anniversary of the University, one of us found *Lotus corniculatus* growing in several places. A plant of somewhat rank growth occurring in grass of rank growth at the roadside near Largoward, Fife, did not afford cyanide but this was detected in a plant of less luxuriant growth found in the same locality in short grass bordering a carriage drive. An extraordinarily dwarf form of the plant was found growing on the sea face of the sand dunes bordering the St. Andrews Golf Links; hydrogen cyanide was not detected in this specimen.

This and last year, it is well known, afford a most remarkable contrast, as in the two seasons the weather has been of very different and opposite types—wet, cold, dull weather having prevailed during the summer of last year (1910), whilst this year (1911) has been characterised by long-continued drought, accompanied by high temperatures and an altogether unusual amount of sunshine.

Our home experience would lead us to correlate the appearance in *Lotus corniculatus* of the cyanophoric glucoside and the attendant enzyme with conditions such as have prevailed during the present year—with conditions favouring maturity rather than luxuriance of growth. But apparently some allowance should be made for a factor of variability, which perhaps is Mendelian, on account of differences observed even during the present phenomenal summer in plants growing under conditions which appear to be very similar if not identical.

In this connection, the following account given of *Lotus corniculatus* in Bentham and Hooker's 'Handbook of the British Flora' is of interest:—

> *L. corniculatus*, Linn., *Bird's-foot Trefoil.*—Stock perennial, with a long tap-root. Stems decumbent or ascending, from a few inches to near 2 feet long. Leaflets usually ovate or obovate; stipules broader than the others. Peduncles much longer than the leaves. Umbels of from five or six to twice that number of bright yellow flowers; the standard often red on the outside. Calyx-teeth about the length of the tube. Pod usually about an inch long. Seeds globular, separated by a pithy substance, which nearly fills the pod.
>
> In meadows and pastures, whether wet or dry, open or shaded, widely spread over Europe, Russian and Central Asia, the East Indian Peninsula and Australia but not reaching the Arctic Circle. Abundant all over Britain. Flowers the whole summer. It is a very variable species, accommodating itself to very different stations and climates; and some of the races appear so permanent in certain localities as to have been generally admitted as species but in others they run so much into one another as to be absolutely indistinguishable.
>
> The most distinct British forms are:—
>
> (a) *L. uliginosus*, Schk.—Tall, ascending or nearly erect; glabrous or slightly hairy and luxuriant in all its parts, with six to eight flowers in the umbel. Calyx-teeth usually but not always finer and more spreading than in the smaller forms. In moist meadows, along ditches, under hedges and in rich, bushy places. *L. major*, Sm.; *L. pilosus*, Beeke.
>
> (b) *L. crassifolius*, Pers.—Low and spreading, often tufted at the base, glabrous or nearly so, usually with five or six rather large flowers to the umbel. Leaflets broad and often glaucous, especially near the sea, where they become much thicker. In open pastures and on dry, sunny banks.
>
> (c) *L. villosus*, Coss. and Germ.—Like the common variety but covered with long, spreading hairs. In dry, sunny situations, common in Southern Europe but in Britain found only in Kent and Devon.

(d) *L. tenuis*, Waldst and Kit.—Slender and more branched than the common form, with very narrow leaflets. In poor pastures and grassy places, chiefly in South-eastern Europe; rare in Britain, always running much into the common form. *L. decumbens*, Forst.

We have not had an opportunity of testing varieties *b* and *c* but have found what we believe to be the variety distinguished as *tenuis* in the Isle of Wight. This has proved to be particularly rich in cyanide.

At times we have thought that size of leaf and degree of hairiness were in some way correlated with the occurrence of cyanide but this has not proved to be the case. There is, however, very little doubt that, as a rule, the dwarf forms are richer in cyanide and that luxuriance of growth favours the disappearance of cyanide.

During August this year one of us has had the opportunity of testing the plant in many places in Norway in the Bergen and the Christiania districts. It was found growing in profusion on the Island of Holsenöe off Bergen, at Voss on the lake shore and on banks at the roadside, at Os in grass and on the roadside near Norheimsund. It was rampant on the moraines at the foot of the Boium and Suphelle glaciers at Fjaerland (Sogne-fjord) and on the Buer glacier at Odda (Hardanger-fjord). Specimens were also secured at Notodden, at Tinnoset and at Eidvos. Dr. Solberg of the Statens Kemiske Kontrolstation at Trondhjem was so kind as to send us a specimen picked at Charlottenlund near Trondhjem. In no single case could cyanide be detected in the Norwegian plant. Of four specimens tested for enzyme, only two contained an appreciable amount and neither came up to the average English plant in activity.

This result appears to us to be very remarkable, especially when the opinion is taken into account which prevails among botanists that both colour and odour are more highly developed in northern regions where light is active during a greater number of hours than it is in our British region.*

Having given most careful attention to the condition of vegetation generally in Norway during August this year, the opinion one of us formed was that the condition everywhere was distinctly and definitely one of relative immaturity and somewhat exuberant growth wherever the circumstances were such as to favour growth. This was particularly noticeable in red currants and raspberries. These fruits, it is well known, grow to a far larger size in Norway than here but they lack the character of English-grown fruit—they appear to be less acid, less sweet, less flavoured and far more "watery." The final impression left was that the conditions in Norway are such as to favour continued growth rather than ripening. From this point of view, it may be questioned whether the specially brilliant colour of Norwegian flowers—if it be a fact—may not be less a consequence of any direct action of light and more an outcome of the greater supply of the colouring agent conditioned by the longer continuation of growth under northern conditions, the supply not being cut down by the setting in of the ripening process at an early stage. Even in our own climate, flowers are apt to be very brilliant in colour in spring and early summer.

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* The argument is also applied to Alpine plants (*cf.* R. R. C. Nevill, 'Journal of the Royal Horticultural Society,' October, 1911, vol. 37, p. 77).

Fortunately we have been able to extend our observations this year practically over the whole of Europe. Dr. J. Vargas Eyre, who has been studying the growth of flax on behalf of the Development Commission, has been able to collect and test Lotus for us at a large number of localities. We are greatly indebted to him for the following summary of his observations:—

| Date. | Place. | Situation. | Character of plant. | HCN. |
|---|---|---|---|---|
| | France. | | | |
| July 11 | Dunkirk | Hot sand | Low growing | Trace |
| November 15 | ,, | Amongst grass on sheltered bank | ,, spreading habit | Distinct |
| | Belgium. | | | |
| July 16 | Werelghem | Moderately moist | Moderately luxuriant | None |
| ,, 17 | Courtrai | Warm, damp (? *L. major*) | Very tall (over 2 feet), hollow stem, large leaves | None |
| ,, 18 | Werelghem | Sun scorched railway track | Very stunted | None |
| | Holland. | | | |
| ,, 28 | Hoorn | Moderately moist | Common type, moderately luxuriant | None |
| ,, 30 | Enkhuisen | ,, | ,, large broad leaves | None |
| August 5 | Bolsward and Arum | (1) Sandy, sun scorched cinder track | Very dwarf type, no sign of flowering | None |
| | | (2) Wet ditch | Slender, branched, narrow leaves (? var. *tenuis*) | Yes |
| | | (3) | Moderately luxuriant, found with (2) | None |
| ,, 9 | Arum | Grass bank | Apparently same as (2) | Yes |
| | Germany. | | | |
| ,, 24 | Potsdam | (1) Lawn of new palace | Dwarf habit, large flowers, very small leaves | None |
| | | (2) | Common type | None |
| | Russia. | | | |
| September 2 | Alt-Schwanenburg | Moist | Common form, luxuriant | None |
| ,, 29 | Orel | (1) River bank, moderately damp | Dwarf habit, small narrow leaves, few in number, hollow stem | None |
| | | (2) ,, ,, | Similar to (1) but large amount of foliage | Yes |
| | | (3) ,, ,, | Similar to (1), except leaves were shorter | Yes |

| Date. | Place. | Situation. | Character of plant. | HCN. |
|---|---|---|---|---|
| | RUSSIA—*contd.* | | | |
| October 1 ... | Zmyarka (50 miles S. of Orel) | (1) Moderately moist | Somewhat erect habit, stalky, few narrow pointed leaves, hollow angular stem | Yes |
| | | (2) „ „ | Creeping habit, much branched, many small blunt leaves, hollow round stem | Yes |
| | | (3) „ „ | Sturdy, woody growth, very small flowers, long narrow drooping leaves | None |
| | | (4) „ „ | Succulent plant, broad leaf, large blossom | Trace |
| | | (5) „ „ | Succulent, abundant large broad leaves, creeping habit | None |
| „ 2 ... | Orel .................. | (1) Upland fields ...... | Bushy, much foliage, small narrow leaves | Yes |
| | | (2) ........................ | Large, stalky growth, few rather broad leaves | Yes |
| | | (3) ........................ | Dwarf habit, very small pointed leaves, much foliage | Yes |
| | | (4) ........................ | Large flowers, dwarf plant, very small pointed leaf | Yes |
| | | (5) ........................ | Large growth, long leaf, large seed pods | Yes |
| | | (6) ........................ | Dwarf habit, broad ended leaf, large flowers | Yes |
| „ 4 ... | Moscow ............ | (1) Damp garden ...... | From E. Russian clover seed, very luxuriant, hollow stem, large long leaves (? *L. major*) | None |
| | | (2) „ ......... | From American clover seed, less luxuriant than (1), full stem, smaller pointed leaves | Yes |
| | | (3) „ ......... | From S. Russian clover seed, full stem, fewer blunt ended leaves, more dwarf habit | Trace |
| | AUSTRIA. | | | |
| „ 11 ... | Trautenau ......... | (1) Kapellenberg, 3000 feet | Small leaf, dwarf plant, much branched, full stem | Yes |
| | | (2) „ „ | More luxuriant than (1) but dwarf, large leaves, full stem | Yes |
| | | (3) „ „ | Small, creeping, narrow leaf ............... | Yes |
| | | (4) „ „ | „ „ small leaf, dark stems... | None |
| | HUNGARY. | | | |
| „ 23 ... | Szeged ............... | (1) Open field ......... | Rather small leaves, thin stems, creeping, much branched plant, somewhat hairy, leaves glaucus | Yes |
| | | (2) „ ......... | Leaves very narrow and sharp pointed, rather hairy plant, large stems, upright | None |
| | ITALY. | | | |
| November 2 | Bologna ............ | (1) Botanical garden... | Weak growth, angular stems, smooth plant, small narrow leaves (*L. tenuis*) | Very distinct |
| | | (2) Three miles S. of Bologna | Small growth, smooth plant, small narrow leaves, luxuriant | Yes |
| | | (3) ........................ | Similar to (2) but more luxuriant growth | Yes |
| | | (4) ........................ | Similar to (3) but much more luxuriant, in warm, moist situation | Yes |
| „ 6 | Florence ............ | (1) Hills about 1½ miles from Florence | Fresh growth from old root, pointed leaves of moderate size | Faint |
| | | (2) „ „ | Similar to (1) but leaf round ended ... | Distinct |
| | | (3) „ „ | „ „ smaller growth......... | Faint |
| | | (4) „ „ | „ (2) but „ ......... | Distinct |

It is probable that, in some cases, the plant Dr. Eyre examined was the *major* variety [Courtrai, Moscow (1)]. On the whole, his observations confirm those we have made at home and in Norway; those which he made in Moscow at the garden of the Agricultural Experiment Station may be referred to as specially interesting: in order to bring under the notice of students the manner in which weeds are introduced with agricultural seeds, neighbouring plots there had been sown with clover seed from various localities. *Lotus corniculatus* was among the weeds on each of the plots but specimens from three plots behaved quite differently: no cyanide was present in the plant—probably the *major* variety—growing on the plot sown with seed from East Russia but that from the plot sown with American seed was extraordinarily rich in cyanide, whilst that derived from seed from South Russia contained but little cyanide. The East Russian plant had a very large leaf; the American plant, rich in cyanide, had the smallest leaf. It may be mentioned that Dr. Eyre could not find *Lotus corniculatus* in the St. Petersburg district.

It is clear that *Lotus corniculatus* is a variable plant, though more often than not, during the present year, it has contained hydrogen cyanide everywhere except in Norway, where the climatic conditions are undoubtedly somewhat special.

Taking into account the variability of the plant and the occurrence of cyanophoric and acyanophoric plants in close proximity, there is reason to suspect crossing and it will be important to carry out experiments from this point of view. But it will be necessary to isolate the varieties first and study these apart. We have secured seed this year in Norway as well as from other places and hope that we shall be able to raise plants next year which will make it possible to state whether the non-occurrence of cyanide in the Norwegian plant is in any way a consequence of climatic influences.

As to the nature of the glucoside present in *Lotus corniculatus:* we have detected acetone as well as hydrogen cyanide and taking into account the fact that the Lotus enzyme acts so readily on linamarin, it is highly probable that the glucoside present is *linamarin*—the glucoside characteristic of many varieties of flax—and that the enzyme is the *linase* which is associated with linamarin in flax. In this connection, the following observations are of interest:—

| | | Percentage activity of enzyme towards | | | |
|---|---|---|---|---|---|
| | | Linamarin. | Prunasin. | Amygdalin. | Salicin. |
| *Lotus corniculatus*... | July 10, 1910 | 64·5 | 32·0 | 2·7 | 27·8 |
| „ *major* ........ | August 10 „ | 1·8 | 2·0 | 1·5 | — |

Unfortunately, we have not yet been successful in our attempts to isolate the glucoside: either the amount present has been too small for us to separate it from the large amount of other substances which are extracted with it or we have been unfortunate in our choice of method.

The following typical determinations of hydrogen cyanide obtainable from the plant may be quoted as showing that the amount present is but small:—

| Source and date (1911). | | Per cent. HCN. |
|---|---|---|
| Portrush. May 22 | | 0·055 |
| Ballantrae. June 7 | | 0·017 |
| Rothamsted (Wild). June 10 | | 0·019 |
| Rothamsted (grown on the barley plots from French seed) | 1A. July 24 | 0·049 |
| | 2A. ,, | 0·044 |
| | 3A. ,, | 0·037 |
| | 4A. ,, | 0·030 |
| Lewisham—Kimmeridge. September 28 (grown at Lewisham in 1911 from seed collected at Kimmeridge in 1910) | | 0·048 |

Taking the highest value (0·055 per cent.), at most about 0·5 per cent. of linamarin can have been contained in the plants examined by us. Unfortunately, in drying the plant, even when this is done very carefully and rapidly at a low temperature, much of the glucoside is destroyed. It will be desirable therefore to work with the undried plant in future.

To determine the enzymic activity of the plant we usually expose it to the action of toluene and afterwards dry it rapidly, *in vacuo*, over oil of vitriol. The dried material is then finely ground. To carry out the determination, 20 c.c. of an M/5 solution of linamarin (0·9886 grm.) is digested with 0·2 grm. of the leaf material during 24 hours at 37°. The amount of hydrogen cyanide liberated is then determined in the manner described in No. XIII of our "Studies on Enzyme Action."

The typical results given on p. 481 may be quoted in illustration.

From the experience gained during the past year, it is clear that in future it will be necessary, in all cases in which hydrogen cyanide is not detected, to test for enzyme as well, so as to discriminate between plants in which both cyanide and enzyme are present and those in which enzyme is present but no cyanide—and to ascertain whether these latter alone have temporarily lost the power of storing the cyanide.

| | Source and date. | Per cent. |
|---|---|---|
| *L. corniculatus* | Thames Bank, July 18, 1910 | 64·5 |
| *L. major* | Swanage, August, 1910 | 1·8 |
| *L. corniculatus* | Portrush, May 22, 1911 | 94·5 |
| „ | Ballantrae, June 28, 1911 | 38·8 |
| „ | Rothamsted (Wild), June 10, 1911 | 92·5 |
| „ | Threshfield, Yorkshire, June 27— | |
| | Small leaves | 82·5 |
| | Large „ | 82·75 |
| „ | Lewisham (from Vilmorin's seed), July 13, 1911 | 90·5 |
| „ | Courtrai (J.V.E.), July 17, 1911— | |
| | (? var. *L. major*), Hairy | 1·5 |
| | Non-hairy | 1·0 |
| „ | Rothamsted, July 24, 1911— | |
| | From Vilmorin's seed — Field 1A | 83·5 |
| | „ 2A | 84·75 |
| | „ 3A | 85·25 |
| | „ 4A | 81·75 |
| „ | Margate | 82·75 |
| „ | Lewisham (from Kimmeridge seed) | 88·0 |
| „ | Fjaerland, August, 1911 | 48·5 |
| „ | Vik, August, 1911 | 17·3 |
| „ | Notodden, August, 1911 | 0·5 |
| „ | Trondhjem, October, 1911 | 0·6 |
| | Vilmorin's seed | 3·75 |
| *L. major* | Cirencester, July 26, 1911 | 0·5 |
| „ | Lewisham (from Vilmorin's seed), July 29, 1911 | 1·0 |
| *L. tenuis* | Isle of Wight, August, 1911 | 79·75 |

It is well that we should put on record the results obtained on growing *Lotus corniculatus* at Rothamsted on four of the barley plots, 1A–4A, across each of which a row was planted, using seed obtained from Vilmorin of Paris. A first crop was cut on August 3, 1911, a second on September 11:—

| | First crop. | Second crop. | Total. |
|---|---|---|---|
| | grammes. | grammes. | grammes. |
| 1A | 464 | 360 | 824 |
| 2A | 458 | 205 | 663 |
| 3A | 541 | 532 | 1073 |
| 4A | 1349 | 774 | 2123 |

Each of the plots receives a dressing of 200 lbs. of ammonia salts per acre. Plot 1A has no other manure; 2A receives 3·5 cwt. superphosphate; 3A 200 lbs. sulphate of potash, 100 lbs. sulphate of soda and 100 lbs. sulphate of magnesia; 4A has a complete mineral manure, receiving both superphosphate and alkali salts.

It is obvious that growth is favoured by complete manuring. The smaller difference between the plots at the second cutting was probably a result of the exceptionally dry condition of the season. The low yield on 2A is perhaps the consequence of the excessive removal of alkalis from the soil in previous years owing to the relative abundance of phosphate.

Lastly, to deal with the value of *Lotus corniculatus* as a forage plant: Mr. R. G. Stapledon, of the Royal Agricultural College, Cirencester, in an interesting report on the Flora of certain Cotswold pastures, published in the 'Scientific Bulletin' of the College (No. 2, 1910, pp. 29—46), makes the following statement in an appendix to his report:—

> Stebler includes *Lotus corniculatus* amongst "the best forage plants." It is indigenous to all parts of England and thrives on all kinds of soil; it is common alike on moors, poor dry land at high elevations and on sandy maritime golf-links. It contributes very largely to the keep of sheep which graze and thrive excellently on the stunted pastures of the latter situations. The sward of the "burrows" by the River Torridge in Devon may be given as an example. . . . The Leguminosæ are represented by *Lotus corniculatus;* the variety *L. crassifolius* is also common.
>
> Prof. T. H. Middleton, in his paper on "The Improvement of Poor Pastures,"* mentions *Lotus corniculatus* as responding well to basic slag, although, as he says, it is not such a good "soil improver" as *Trifolium repens.*

*Lotus corniculatus* is referred to in Sutton's 'Permanent and Temporary Pastures' as a very useful cropper; it is described as standing the severest drought and as able to grow on very light soils and even on clover-sick soils.

*Lotus major*, for some reason which is not apparent, is spoken of as inferior to *Lotus corniculatus.* Prof. Percival, in his 'Agricultural Botany,' refers to *Lotus corniculatus* seed as being usually adulterated with worthless *Lotus major* seed.

It will be of great importance not only to ascertain whether *Lotus corniculatus* is a valuable forage plant but to what extent, if at all, its value is to be correlated with the presence of the cyanophoric glucoside. Should it be ascertained that the glucoside is of special value, it will probably not be difficult to introduce into pastures the variety of the plant which is likely to be of maximum value. A step will also have been taken towards solving the problem to which we have called attention at the outset of this communication, as a method will have been devised which will be applicable to the study of other plants.

We have naturally turned our attention to other species of Lotus. We did not find hydrogen cyanide in *Lotus tetragonolobus* growing in the Saas valley in August last year nor in plants raised this year at Lewisham and Reading from seed supplied by Messrs. Vilmorin. Dr. Eyre has tested *Lotus siliquosus* growing in Bologna this year without finding cyanide. We have also failed in finding the cyanide in plants of *Lotus Bertholetti* (*peliorhynchus*), kindly placed at our disposal by Dr. Hugo Müller and by Mr. R. A. Robertson of St. Andrews University. But in *Lotus Jacobæus*, a native of the Canary Islands, we have found a plant which seems to be as rich both in cyanide and enzyme as *L. corniculatus*. We have raised this plant ourselves from seed. As it also contains acetone and the enzyme acts readily on linamarin, it is probable that the glucoside and enzyme are identical with those occurring in *Lotus corniculatus*. A plant was shown to Dr. Eyre in the Bologna botanic garden as *Lotus corniculatus* which he was informed was grown in Italy as a fodder plant; to judge from the specimen he has sent to us this was *Lotus Jacobæus*. We shall continue the study of this plant during the coming year and hope to be able to test the other species of Lotus not yet examined. It should be added that we have failed to find cyanide in *Hippocrepis comosa*, a plant which resembles *Lotus corniculatus* very closely.

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* 'Journal of Agricultural Science,' vol. 1.

We have to express our thanks for the assistance that has been given to us by Mr. Hall, Director of the Rothamsted Agricultural Experiment Station, by Dr. Keeble, Professor of Botany at the University College, Reading, as well as by many other willing helpers, particularly by Dr. J. V. Eyre, to whom we are so much indebted for the work he did for us abroad.

We desire also to ask botanists under whose notice this communication comes to favour us by carefully testing *Lotus corniculatus* for hydrogen cyanide, at the same time noting any botanical peculiarities the plant may show and the conditions under which it grows. We would ask them to inform us of the results they obtain and to favour us also with specimens whenever peculiarities are noticed. It will be best to cut the root well below ground and to send whole plants; we like to have several grammes of the leaf material, if possible, when testing for enzyme. We shall also be glad to have young plants or seed for further study.

In testing for cyanide we find it most convenient to make use of stout glass tubes, about 3½ inches long and ½ inch wide, provided with good corks. The leaf material having been pushed into the tube, two or three drops of chloroform or toluene are added and a slip of moist picrate paper is inserted; the tube is then corked up. It is conveniently incubated in a waistcoat breast-pocket or in the trousers pocket. When cyanide is present the paper reddens perceptibly within half an hour, as a rule; to make certain, the test should be prolonged over 24 hours. To prepare the picrate paper, slips of filter paper about ⅜ inch wide are dipped into a solution of 5 grm.

picric acid and 50 grm. sodium carbonate in 1 litre of water; after draining the paper, it is hung from a pin to dry until it is only just perceptibly moist; it is then cut up into $\frac{3}{4}$-inch lengths and stored in a closed tube. It is well to keep a piece of the paper in each of the stock of tubes carried, so as to make sure that hydrogen cyanide has not been stored up in the cork.

City and Guilds College,
South Kensington, London.

# *Antelope Infected with* Trypanosoma gambiense.

By Captain A. D. Fraser, R.A.M.C., and Dr. H. L. Duke.

(Communicated by Sir John Bradford, Sec. R.S. Received December 2, 1911,—Read January 25, 1912.)

The Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10, showed that waterbuck, bushbuck and reedbuck could be readily infected with a human strain of *Trypanosoma gambiense*, and that clean laboratory-bred *Glossina palpalis* were capable of transmitting the virus from the infected antelope to susceptible animals.

In the present paper, observations which were made upon these antelope during the eight months subsequent to the Commission's departure from Uganda are recorded. Experiments are also described which show that the duiker—another species of antelope common in most parts of Uganda—can also be similarly infected with a human strain of *T. gambiense*. As regards the antelope employed by the Commission, six of the nine remained in apparently excellent health in April, 1911—roughly, a year after they were infected.

Until Bushbuck 2428 escaped from the kraal, and Bushbuck 2372 died 338 days after its infection as the result of an accident, they had also been healthy. A *post-mortem* examination was made immediately after death in the case of Bushbuck 2372, but no evidence of trypanosomiasis was found.

Reedbuck 2427 appeared to be perfectly healthy for 200 days after it had been infected. It then died suddenly. At the *post-mortem* examination performed immediately after death the prescapular glands were found to be the size of a hazel-nut. On section they were hæmorrhagic. There were numerous petechiæ on the mucous membrane of the mesentery. The mucous membrane of the fourth stomach also showed many petechiæ. Microscopical examination of smears made from the various organs was negative. It is, therefore, impossible to say what the cause of death was in the case of this buck.

With the view of ascertaining how long the antelope remained infected, investigations were carried out on the following lines:—

1. Feeding laboratory-bred *Glossina palpalis* for several days on the antelope and subsequently endeavouring to infect a healthy susceptible animal.
2. Dissecting these flies and examining them for flagellates.
3. Injecting blood of the buck into animals susceptible to *T. gambiense* infection.

Table I gives the detailed results obtained by the first of these methods The number of days which elapsed from the date on which the buck was infected until the commencement of the experiment is given.

"Hereditary transmission" flies indicates that the flies before being put upon the antelope had been fed for 30 days upon healthy monkeys to ascertain if laboratory-bred flies which had never fed upon an infected animal could give rise to an infection. As it has been suggested that flies were most readily infected when their first feed was upon an infected animal, these flies were used with the view of obtaining evidence on this point, control experiments being at the same time made with laboratory-bred flies which had not fed before they were put upon the antelope. Although it will be noted that no infection occurred among the "hereditary transmission" flies, whereas the control flies sometimes became infected, the numbers of the flies used are too small to allow of any conclusions being arrived at.

It will be noted that flies which were fed on Bushbuck 2372, 315 days after it had been infected with a human strain of *T. gambiense,* became infected and successfully transmitted the disease to a healthy monkey.

Table II gives the results of the dissections of laboratory-bred *Glossina palpalis* which had fed on the infected antelope.

The experiments recorded in Tables I and II are summarised and grouped according to the length of time the antelope had been infected in Table III.

Table I.—Giving the Results of Feeding Laboratory-bred *Glossina palpalis* on Antelope infected with *T. gambiense*.

| Experiment No. | No. of flies. | | No. of days after original infection of antelope. | No. of days flies fed on antelope. | No. of days before flies became infective. | Result. | | Length of experiment, in days. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| | On 1st day. | On 30th day. | | | | Positive. | Negative. | | |
| | | | | | Bushbuck, Expt. 2328. | | | | |
| 7 | ? | ? | 134 | 5 | 25 | + | | 32 | Experiment carried out by Dr. van Someren. |
| 215 | 115 | 79 | 202 | 10 | | | – | 79 | |
| 402 | 53 | 18 | 264 | 11 | | | – | 64 | " Hereditary transmission " flies. |
| 403 | 49 | 29 | 264 | 11 | | | – | 64 | Control to Expt. 402. |
| 538 | 21 | 16 | 306 | 7 | | | – | 48 | " Hereditary transmission " flies. |
| 539 | 39 | 31 | 306 | 7 | | | – | 62 | Control to Expt. 538. |
| 647 | 52 | 46 | 342 | 6 | | | – | 41 | |
| | | | | | Bushbuck, Expt. 2371. | | | | |
| 89 | ? | ? | 126 | 3 | 50 | + | | 59 | Experiment carried out by Dr. van Someren. |
| 216 | 90 | 64 | 173 | 10 | | | – | 78 | |
| 404 | 50 | 21 | 235 | 11 | | | – | 63 | " Hereditary transmission " flies. |
| 405 | 43 | 31 | 235 | 11 | 38 | + | | 51 | Control to Expt. 404. |
| 543 | 33 | 17 | 279 | 9 | | | – | 60 | " Hereditary transmission " flies. |
| 544 | 71 | 44 | 279 | 9 | | | – | 60 | Control to Expt. 543. |
| 643 | 73 | 49 | 311 | 5 | | | – | 42 | |
| | | | | | Bushbuck, Expt. 2372. | | | | |
| 88 | ? | ? | 123 | 3 | 39 | + | | 48 | Experiment carried out by Dr. van Someren. |
| 218 | 72 | 59 | 171 | 9 | 34 | + | | 47 | |
| 480 | 79 | 47 | 253 | 7 | | | – | 64 | |
| 607 | 85 | 61 | 298 | 8 | 25 | + | | 33 | |
| 658 | 64 | 48 | 315 | 9 | 38 | + | | 38 | |
| | | | | | Bushbuck, Expt. 2428. | | | | |
| 106 | ? | ? | 116 | 2 | | | – | 67 | Experiment carried out by Dr. van Someren. |
| 222 | 20 | 20 | 164 | 9 | 40 | + | | 49 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Reedbuck, Expt. 2357. | | | | | |
| 51 | 92 | 18 | 131 | 7 | | | — | 77 | |
| 189 | 101 | 62 | 173 | 11 | 44 | + | | 59 | |
| 469 | 83 | 34 | 263 | 9 | 31 | + | | 43 | |
| 528 | 41 | 20 | 288 | 8 | | | — | 57 | " Hereditary transmission " flies. |
| 529 | 70 | 55 | 288 | 8 | 34 | + | | 43 | Control to Expt. 528. |
| 631 | 97 | 56 | 322 | 6 | | | — | 41 | |
| 669 | 119 | 63 | 336 | 12 | | | — | 35 | |
| | | | | Reedbuck, Expt. 2359. | | | | | |
| 52 | 170 | 64 | 113 | 7 | 38 | + | | 55 | |
| 190 | 89 | 76 | 155 | 11 | 32 | + | | 44 | |
| 398 | 50 | 34 | 231 | 12 | 36 | + | | 49 | Control to Expt. 400. |
| 400 | 49 | 13 | 231 | 12 | | | — | 63 | " Hereditary transmission " flies. |
| 530 | 54 | 19 | 270 | 8 | | | — | 61 | |
| 642 | 33 | 29 | 306 | 5 | | | — | 40 | |
| | | | | Reedbuck, Expt. 2427. | | | | | |
| 97 | 60 | 53 | 117 | 8 | 34 | + | | 46 | |
| 254 | 72 | 52 | 177 | 11 | 29 | + | | 49 | |
| | | | | Reedbuck, Expt. 2431. | | | | | |
| 98 | 81 | 41 | 119 | 7 | 32 | + | | 43 | |
| 268 | 60 | 51 | 184 | 10 | 36 | + | | 50 | |
| 399 | 43 | 29 | 224 | 12 | | | — | 63 | Control to Expt. 401. |
| 401 | 25 | 4 | 224 | 12 | | | — | 63 | " Hereditary transmission " flies. |
| 598 | 88 | 44 | 288 | 9 | | | — | 50 | |
| 656 | 66 | 30 | 306 | 11 | | | — | 40 | |
| | | | | Waterbuck, Expt. 2378. | | | | | |
| 6 | ? | ? | 105 | 5 | 25 | + | | 32 | Experiment carried out by Dr. van Someren. |
| 217 | 168 | 53 | 173 | 10 | | | — | 68 | |
| 406 | 54 | 0 | 237 | 9 | | | — | 19 | |
| 471 | 96 | 9 | 251 | 7 | | | — | 62 | |
| 488 | 45 | 28 | 259 | 8 | | | — | 59 | |
| 523 | 36 | 24 | 270 | 7 | | | — | 62 | |
| 622 | 62 | 15 | 306 | 7 | | | — | 42 | |

Table II.—Giving the Results of the Dissection of Laboratory-bred *Glossina palpalis* which had Fed on Antelope infected with *T. gambiense.*

| Experiment No. | No. of flies used in experiment. | No. of flies dissected. | No. of infected flies found. | Percentage of infected flies. | Result of experiment. | Remarks. |
|---|---|---|---|---|---|---|
| Bushbuck, Expt. 2328. | | | | | | |
| 7 | ? | 0 | 0 | | + | Flies not dissected. |
| 215 | 115 | 79 | 0 | | — | |
| 402 | 53 | 42 | 0 | | — | |
| 403 | 49 | 42 | 0 | | — | |
| 538 | 21 | 21 | 0 | | — | |
| 539 | 39 | 33 | 0 | | — | |
| 647 | 52 | 51 | 0 | | — | |
| Bushbuck, Expt. 2371. | | | | | | |
| 89 | ? | 27 | 0 | | + | |
| 216 | 90 | 61 | 0 | | — | |
| 404 | 50 | 33 | 0 | | — | |
| 405 | 43 | 41 | 0 | | + | |
| 543 | 33 | 26 | 0 | — | — | |
| 544 | 71 | 66 | 0 | | — | |
| 643 | 73 | 51 | 0 | | — | |
| Bushbuck, Expt. 2372. | | | | | | |
| 88 | ? | 29 | 1 | 3·45 | + | |
| 218 | 72 | 37 | 1 | 2·7 | + | |
| 480 | 79 | 44 | 0 | | — | |
| 607 | 84 | 70 | 1 | 1·4 | + | |
| 658 | 64 | 59 | 2 | 3·4 | + | |
| 356 | 36 | 36 | 0 | | — | Experiment lasted 12 days. |
| 357 | 38 | 38 | 0 | | — | „ „ 14 „ |
| Bushbuck, Expt. 2428. | | | | | | |
| 106 | ? | 14 | 0 | | — | |
| 222 | 20 | 20 | 2 | 10 | + | |
| Reedbuck, Expt. 2357. | | | | | | |
| 51 | 92 | 30 | 0 | | — | |
| 189 | 101 | 46 | 4 | 8·6 | + | |
| 469 | 83 | 53 | 5 | 9·4 | + | |
| 528 | 41 | 31 | 0 | | — | |
| 529 | 70 | 64 | 3 | 4·7 | + | |
| 631 | 97 | 77 | 0 | | — | |
| 669 | 119 | 65 | 0 | | — | |
| Reedbuck, Expt. 2359. | | | | | | |
| 52 | 170 | 122 | 0 | | + | |
| 190 | 89 | 61 | 5 | 8·1 | + | |
| 398 | 50 | 49 | 3 | 6·1 | + | |
| 400 | 49 | 40 | 0 | | — | |
| 530 | 54 | 23 | 0 | | — | |
| 642 | 33 | 30 | 0 | | — | |
| Reedbuck, Expt. 2427. | | | | | | |
| 97 | 60 | 27 | 1 | 3·7 | + | |
| 254 | 72 | 49 | 1 | 2·0 | + | |
| 322 | 28 | 28 | 0 | | — | Experiment lasted 9 days. |
| 323 | 30 | 30 | 1 | 3·3 | — | „ „ 13 „ |

Table II—*continued.*

| Experiment No. | No. of flies used in experiment. | No. of flies dissected. | No. of infected flies found. | Percentage of infected flies. | Result of experiment. | Remarks. |
|---|---|---|---|---|---|---|
| Reedbuck, Expt. 2431. | | | | | | |
| 98 | 81 | 8 | 0 | | + | |
| 268 | 60 | 53 | 2 | 3·7 | + | |
| 399 | 43 | 36 | 0 | | — | |
| 401 | 25 | 14 | 0 | | — | |
| 598 | 88 | 45 | 0 | | — | |
| 656 | 66 | 35 | 0 | | — | |
| Waterbuck, Expt. 2378. | | | | | | |
| 6 | ? | 0 | 0 | | + | |
| 217 | 168 | 53 | 2 | 3·8 | — | |
| 406 | 54 | 36 | 0 | | — | |
| 471 | 96 | 18 | 0 | | — | |
| 488 | 45 | 44 | 0 | | — | |
| 523 | 36 | 31 | 0 | | — | |
| 622 | 62 | 20 | 0 | | — | |

Table III.—Summarising Experiments of Tables I and II and grouping them according to Length of Time the Antelope had been infected.

| Species of antelope. | Days after original infection of antelope. | No. of experiments. | No. of positive experiments. | No. of flies. | | No. of flies dissected. | No. of infected flies found. |
|---|---|---|---|---|---|---|---|
| | | | | On 1st day. | On 30th day. | | |
| Bushbuck 2328 ... | 100—200 | 1 | 1 | ? | ? | 0 | 0 |
| „ 2371 ... | „ | 2 | 1 | ? | ? | 88 | 0 |
| „ 2372 ... | „ | 2 | 2 | ? | ? | 66 | 2 |
| „ 2428 ... | „ | 2 | 1 | ? | ? | 34 | 2 |
| Reedbuck 2357 ... | „ | 2 | 1 | 193 | 80 | 76 | 4 |
| „ 2359 ... | „ | 2 | 2 | 259 | 140 | 183 | 5 |
| „ 2427 ... | „ | 4 | 2 | 190 | 105 | 134 | 3 |
| „ 2431 ... | „ | 4 | 2 | 209 | 125 | 111 | 2 |
| Waterbuck 2378... | „ | 2 | 1 | ? | ? | 53 | 2 |
| Bushbuck 2328 ... | 200—300 | 3 | 0 | 217 | 126 | 163 | 0 |
| „ 2371 ... | „ | 4 | 1 | 197 | 113 | 166 | 0 |
| „ 2372 ... | „ | 4 | 1 | 238 | 108 | 188 | 1 |
| Reedbuck 2357 ... | „ | 3 | 2 | 194 | 118 | 148 | 8 |
| „ 2359 ... | „ | 3 | 1 | 151 | 66 | 112 | 3 |
| „ 2431 ... | „ | 2 | 0 | 154 | 74 | 80 | 0 |
| Waterbuck 2378... | „ | 4 | 0 | 231 | 61 | 129 | 0 |
| Bushbuck 2328 ... | 300—342 | 3 | 0 | 112 | 93 | 105 | 0 |
| „ 2371 ... | „ | 1 | 0 | 73 | 49 | 51 | 0 |
| „ 2372 ... | „ | 1 | 1 | 64 | 48 | 59 | 2 |
| Reedbuck 2357 ... | „ | 2 | 0 | 216 | 119 | 142 | 0 |
| „ 2359 ... | „ | 1 | 0 | 33 | 29 | 30 | 0 |
| Waterbuck 2378... | „ | 1 | 0 | 62 | 15 | 20 | 0 |

It will be seen that positive experiments were obtained from all the buck (nine examined) when the flies were fed upon them before 200 days had elapsed from the date of the antelope's infection. When more than 200 days had elapsed four of the seven buck examined yielded positive results.

The results of all experiments are shown in Table IV.

Table IV.—Giving Results of Experiments from all Antelopes.

| Interval in days after infection of antelope. | No. of experiments. | No. of positive experiments. | No. of flies dissected. | No. of flies found infected. | Percentage of infected flies. |
|---|---|---|---|---|---|
| 100—200 | 21 | 13 | 745 | 20 | 2·7 |
| 200—300 | 23 | 5 | 986 | 12 | 1·2 |
| 300—342 | 9 | 1 | 407 | 2 | 0·5 |
| Totals ............ | 53 | 19 | 2138 | 34 | 1·6 |

It appears from the above table that as the interval after the infection of the antelope increases, the percentage of positive transmission experiments and of flies which become infected with flagellates after having fed on the buck diminishes. This diminution becomes still more striking when these results are compared with those recorded by the Commission of experiments carried out soon after the antelope were infected. (Of the 24 experiments carried out by the Commission 17 were positive, 1722 flies were dissected, and 6·9 per cent. were found to be infected.)

The results of injecting blood of these antelope into susceptible animals are shown in Table V (p. 491).

It is seen that the injection of a small quantity of the blood of Bushbuck 2372, 327 days after it had been infected with *T. gambiense*, produced an infection in a white rat. This, however, was the only positive result which was obtained. Three injections were carried out from Waterbuck Experiment 2378—on one occasion 5 c.c. of blood was injected—and all were negative. It will be remembered that the Commission found it easy to produce infections in susceptible animals by injecting the blood taken from these antelope soon after they were infected.

Table V.—Giving Results of injecting the Blood of Antelope infected with *T. gambiense* into susceptible Animals.

| Antelope. | No. of days after infection of antelope. | Animal used. | Quantity of blood injected in c.c. | Result. | Remarks. |
|---|---|---|---|---|---|
| Reedbuck 2427 ... | 200 | Monkey | 2 | Inconclusive | Monkey died. Negative for 9 days. |
| Waterbuck 2378... | 306 | White rat | 1 | — | |
| " " ... | 316 | Monkey | 5 | — | |
| " " ... | 316 | White rat | 1 | — | |
| Bushbuck 2328 ... | 355 | " | 1½ | — | |
| " 2371 ... | 330 | " | 1 | — | |
| " 2372 ... | 327 | " | 1 | + | Trypanosomes appeared in rat on the 12th day. |
| Reedbuck 2357 ... | 345 | " | 1 | — | |
| " 2359...... | 327 | " | 1 | — | |
| " 2431...... | 320 | " | 1 | — | |
| Bushbuck 2372 ... | 338 | Monkey | 5 | Inconclusive | Monkey died before trypanosomes could have appeared. |

### *Can a Duiker be Infected with a Human Strain of* T. gambiense ?

*Experiment* 99, *Duiker.*—On August 30, 1910, 3 c.c. of this buck's blood were injected subcutaneously into a normal monkey to ascertain if the antelope naturally harboured trypanosomes. The monkey's blood was examined regularly for a month. No trypanosomes appeared in its blood, the monkey remaining healthy.

For nine days (January 25 to February 2, 1911, inclusive) laboratory-bred *G. palpalis* known to be infected with a human strain of *T. gambiense* were fed upon the buck.

On February 4, the tenth day after the infected flies first fed upon the antelope, *T. gambiense* appeared in fair numbers in its blood.

On February 10 and 11, 1911, 119 clean laboratory-bred *G. palpalis* were fed upon the duiker. These flies were subsequently fed on a normal monkey, which they infected after 28 days had elapsed from the date of their first feed on the buck. Of 42 flies which were dissected, two were found to be infected with flagellates.

*Remarks.*—The duiker was free from trypanosomes inoculable into a monkey on its arrival at the laboratory.

*T. gambiense* appeared in the buck's blood on the tenth day after infected flies had fed upon it, and clean laboratory-bred flies successfully transmitted the infection to a healthy susceptible monkey.

### *Conclusions.*

1. Antelope may remain in apparently perfect health for a year after having been infected with a human strain of *T. gambiense.*

2. One antelope was still capable of infecting clean laboratory-bred *G. palpalis* 315 days after it had been infected.

3. A small quantity of blood taken from one antelope 327 days after its infection was proved by inoculation into a white rat to be infective.

4. As the interval after the infection of the antelope increases their infectivity, as tested by "cycle" transmission experiments, dissection of flies which have fed upon them, and by the injection of the buck's blood into susceptible animals, appears to diminish.

5. A duiker was infected with a human strain of *T. gambiense* by feeding infected *G. palpalis* upon it.

# *The Bacterial Production of Acetylmethylcarbinol and 2.3-Butylene Glycol from Various Substances.*

By Arthur Harden, F.R.S., and Dorothy Norris.

(Received November 22, 1911,—Read February 1, 1912.)

(From the Biochemical Department, Lister Institute.)

In working out the action of *B. lactis aërogenes* on glucose quantitatively, Harden and Walpole (1) found that, in addition to the products already noted in the action of *B. coli communis* on glucose (2), a small quantity of acetylmethylcarbinol, $CH_3{\cdot}CH(OH){\cdot}CO{\cdot}CH_3$, and a considerable proportion of 2.3-butylene glycol, $CH_3{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_3$, were formed, the latter corresponding to about 33 per cent. of the carbon of the sugar fermented. The production of acetylmethylcarbinol by the action of *Tyrothrix tenuis, B. subtilis* and *B. mesentericus vulgatus,* and similar organisms on glucose, had been previously noted by Grimbert (3) and by Desmots (4).

The presence of acetylmethylcarbinol is of especial interest, as it has been shown to be the substance responsible for the Voges and Proskauer reaction (5). In view of this fact, and of the interest attaching to this mode of decomposition of glucose, it becomes a matter of some importance to discover what substances will give rise to acetylmethylcarbinol and butylene glycol during fermentation, and also which bacteria are capable of producing these two compounds. *B. lactis aërogenes* and *B. cloacæ* have been shown to produce both substances from glucose, mannitol, and fructose (6, 7, 8); hence, in the first place, the action of these organisms was studied, and similar experiments were later carried out with *B. coli communis*, but with negative results.

It was of especial interest to discover whether these substances could be produced from carbon compounds less complex than the hexoses, and a variety of simpler substances containing fewer carbon atoms were therefore tried, *e.g.*, glycerol, ethylene glycol, malic acid, etc.

The formation of acetylmethylcarbinol or butylene glycol from substances containing three or fewer carbon atoms would necessarily involve a carbon synthesis which would be of considerable theoretical interest. An instance of this kind, the production of butyric acid and butyl alcohol from lactic acid and from glycerol in the butyric fermentation, has long been known (9, 10). To explain this, it has been supposed that acetaldehyde is first formed and then serves as the source of the butyl alcohol and butyric acid. The aldehyde may be supposed to undergo an aldol condensation followed by reduction, with or without subsequent oxidation:

$$2CH_3{\cdot}CHO = CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CHO.$$

$$CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CHO + 4H = CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}OH + H_2O.$$

It seems possible that the production of acetylmethylcarbinol and butylene glycol may be due to a somewhat similar course of events, which may be represented as follows, although these reactions have not, hitherto, been observed in the laboratory :—

$$2CH_3{\cdot}CHO + 2H = CH_3{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_3.$$

$$2CH_3{\cdot}CHO = CH_3{\cdot}CO{\cdot}CH(OH){\cdot}CH_3.$$

Experiments to test this hypothesis were made, with the result that the production of the glycol by bacterial action from acetaldehyde was conclusively established, although the mechanism of this production has not yet been ascertained.

It by no means necessarily follows, however, that in the fermentation of glucose the butylene glycol is actually produced from preformed acetaldehyde.

### 1. *Experimental Methods.*

As a general rule the culture medium consisted of 1 per cent. Witte's peptone water containing 5 per cent. of the substance under investigation. Sufficient chalk was added to neutralise any acid formed during fermentation, and after inoculation the culture was grown for three weeks at 37° C. under anaërobic conditions, the flask being fitted with a mercury trap to allow the escape of any gases.

Among the products of the reaction, acetylmethylcarbinol and butylene glycol only have been estimated quantitatively. A more complete investigation has, however, been made in the case of glycerol, and the results obtained will form the subject of another communication.

To ascertain whether the carbinol and glycol were derived from the acetaldehyde, sugar or other substances in question, and not simply from a constituent of the peptone, control experiments were carried out with peptone water alone as culture medium. In no case could the slightest trace of either substance be detected.

### 2. *Detection and Estimation of Acetylmethylcarbinol and Butylene Glycol.*

As some of the substances employed could only be used in small quantities, it was necessary to elaborate a method for the detection of both acetylmethylcarbinol and butylene glycol in small amounts. Attempts were first made to separate these two compounds by cautious evaporation and estimate them individually, but it was found impossible to arrive at any quantitative values in this way.

The method ultimately adopted was as follows :—

The medium in which the organism had been grown was carefully distilled to as small a bulk as possible over a free flame and then to dryness under reduced pressure at 37° C. The distillates were then united and made up to a definite volume and portions tested for reducing power and for Voges and Proskauer's reaction, which, as stated above, is due to the presence of acetylmethylcarbinol. To perform this reaction 3 c.c. of 1 per cent. Witte's peptone water are mixed in a test-tube with an equal quantity of 10 per cent. caustic soda, 2 c.c. of the solution to be tested are then carefully poured on to the surface of the liquid, and the tube is allowed to stand at room temperature. If acetylmethylcarbinol be present, a pink ring forms at the juncture of the two liquids. With very small quantities of carbinol, this may take some hours to develop, but with larger amounts the colour soon appears and quickly spreads through the whole of the solution, a green fluorescence being also produced. It was found that more delicate results could be obtained by the above method than by simply mixing the solutions.

Experiments in connection with other work, shortly to be published, have indicated that the carbinol is alone responsible for the reducing power of the distillates, and hence an estimation of this by Bang's (11) method at once gives the amount of acetylmethylcarbinol present, the reducing power of this substance being known (12).

The estimation of the butylene glycol is not such a simple matter.

A small amount is held in the dry residue after distillation, and must be extracted with dry ether, and the glycol thus obtained, after evaporation of the ether, added to the distillate.

The estimation depends on the fact that the glycol is readily oxidised by bromine under the influence of light to diacetyl, which can be estimated by its reducing power. Any acetylmethylcarbinol present is quantitatively oxidised to diacetyl in this way, but as the quantity of this substance will already have been determined, the amount of diacetyl formed from it is known, and the difference between this and the total diacetyl represents that due to the glycol. The details of the process are as follows:—An aliquot portion of the distillate is treated with a small quantity of bromine (0·1 c.c. for the distillate from the fermentation of 5 grm. of substance), and left exposed for 12 to 15 hours to the light of a 50-candle-power electric lamp. A further addition of bromine is made if the solution becomes completely decolorised, and the exposure continued. Any free bromine is then removed by the cautious addition of sulphurous acid, excess of this being carefully avoided. The solution is next saturated with sodium chloride, and the diacetyl distilled off and estimated by determining the reducing power of the distillate.

The results obtained by the above method are low, as the oxidation of the glycol to diacetyl is not quantitative. Control experiments with known amounts of glycol show that the actual results obtained are two-thirds the correct value, and a correction for this has been made in the tables given below.

In most cases the diacetyl produced by the oxidation was further characterised by the preparation and analysis of the phenylosazone.

Two typical analyses gave the following results:—

1. Substance fermented—galactose. Organism—*B. cloacæ.* Osazone—m.p. 243° C.

0·2109 grm. gave 39·4 c.c. N at 23·5° C. and 765 mm. N = 21·14 per cent.

2. Substance fermented—arabinose. Organism—*B. lactis aërogenes.* Osazone—m.p. 244° C.

0·1349 grm. gave 25 c.c. N at 22° C. and 765 mm. N = 21·13 per cent.
Diacetylphenylosazone, $C_{16}H_{18}N_4$, requires N = 21·05 per cent.

In the sugar experiments the amount unfermented was estimated in the residue left after the extraction of the glycol by dissolving it in water, making up to a known volume, and determining the reducing power after treatment with mercuric nitrate (Patein).

### 3. *Results Obtained.*

The results obtained with the organisms employed are indicated in the tables given below. The figures given in Columns 8 and 9 are the values calculated from the actual results found for 10 grm. of sugar fermented.

As previously mentioned, the results obtained with *B. coli communis* were in every case negative.

Table I.—Action of *B. lactis aërogenes* (Escherich) and *B. cloacæ* (Jordan) on various Sugars.

| Experiment. | Organism. | Sugar. | Weight fermented. | Time of growth. | Carbinol per 10 grm. sugar fermented. | Glycol per 10 grm. sugar fermented. |
|---|---|---|---|---|---|---|
| | | | grm. | | grm. | grm. |
| 1 | *B. lactis aërogenes* | Glucose | 4·66 | 3 weeks | 0·11 | 1·42 |
| 2 | „ „ | „ | 4·85 | „ | 0·12 | 1·39 |
| 3 | „ „ | „ | 5·00 | „ | 0·11 | 1·36 |
| 4 | „ „ | „ | 2·47 | „ | 0·11 | 1·44 |
| 5 | *B. cloacæ* | Fructose | 4·67 | „ | 0·11 | 1·55 |
| 6 | „ | „ | 4·15 | „ | 0·11 | 1·51 |
| 7 | „ | Mannose | 2·32 | „ | 0·11 | 1·36 |
| 8 | *B. lactis aërogenes* | „ | 2·45 | „ | 0·10 | 1·42 |
| 9 | „ „ | Galactose | 4·03 | „ | 0·06 | 0·86 |
| 10 | „ „ | „ | 2·40 | „ | 0·07 | 0·85 |
| 11 | *B. cloacæ* | „ | 4·74 | „ | 0·08 | 1·41 |
| 12 | „ | „ | 4·38 | „ | 0·08 | 1·36 |
| 13 | *B. lactis aërogenes* | Arabinose | 3·92 | „ | 0·07 | 1·18 |
| 14 | „ „ | „ | 2·39 | „ | 0·08 | 1·19 |
| 15 | *B. cloacæ* | „ | 5·00 | „ | 0·06 | 1·19 |
| 16 | „ | „ | 1·83 | „ | 0·08 | 1·18 |
| 17 | *B. lactis aërogenes* | Isodulcite | 2·03 | 32 days | 0·43 | 1·45 |
| 18 | „ „ | „ | 1·77 | 3 weeks | 0·67 | 1·54 |
| 19 | *B. cloacæ* | „ | 2·03 | „ | 0·04 | 0·81 |

It will be seen from the above table that glucose, fructose, and mannose have given practically the same quantities of carbinol and glycol respectively, whilst with galactose the carbinol is slightly less in amount with both organisms used, and the yield of glycol varies with the organism employed. The results obtained in the case of arabinose show close agreement for both organisms, although in all four cases lower figures are obtained than with the hexoses. The question of isodulcite is interesting, as the amount of carbinol obtained by means of *B. lactis aërogenes* is abnormally high compared with that obtained from the other sugars or by using *B. cloacæ*.

The galactose and arabinose used in the above experiments were previously purified from traces of glucose by fermentation with yeast.

The amount of glycol produced from glucose is decidedly lower than that obtained by Harden and Walpole, a result which is possibly due to the employment of a different strain of the organism.

Table II.—Action of *B. lactis aërogenes* (Escherich) and *B. cloacæ* (Jordan) on various Alcohols.

| Experiment. | Organism. | Alcohol. | Weight taken. | Carbinol per 10 grm. taken. | Glycol per 10 grm. taken. |
|---|---|---|---|---|---|
| | | | grm. | grm. | grm. |
| 20 | *B. lactis aërogenes* | Glycerol | 5 | Nil | 0·05 |
| 21 | „ „ | „ | 50 | „ | 0·05 |
| 22 | „ „ | „ | 10 | „ | 0·05 |
| 23 | „ „ | „ | 10 | „ | 0·04 |
| 24 | *B. cloacæ* | „ | 10 | „ | Nil |
| 25 | *B. lactis aërogenes* | Ethylene glycol | 5 | „ | 0·08 |
| 26 | „ „ | „ | 5 | „ | 0·08 |
| 27 | „ „ | „ | 5 | „ | 0·09 |
| 28 | „ „ | Adonitol | 2·50 | 0·06 | 0·22 |
| 29 | „ „ | „ | 2·50 | 0·06 | 0·24 |
| 30 | „ „ | Mannitol | 5 | 0·03 | 0·23 |
| 31 | „ „ | „ | 5 | 0·03 | 0·04 |

The growth was continued for three weeks, except in the case of Experiment 31, which was for two weeks. The residual alcohols were not estimated, so that the results stated above are not corrected for amount of substance unfermented. Only in the case of mannitol and adonitol was any carbinol detected. All these alcohols, however, yielded glycol. Citric and malic acids gave negative results with both organisms.

The action of *B. lactis aërogenes* on dihydroxyacetone, $CH_2(OH)\cdot CO\cdot CH_2OH$, was also tried, but here again without positive result.

### 4. *Experiments on the Synthesis of* 2.3-*Butylene Glycol from Acetaldehyde by means of* B. lactis aërogenes.

For each experiment a litre of Witte's peptone water was made up and sufficient chalk added to neutralise any acids which might be formed during fermentation. The medium was then inoculated with *B. lactis aërogenes* and incubated at 37° C. for 24 hours before any addition of acetaldehyde was made, in order to establish a good growth of the organism.

In some experiments calcium formate (10 grm. formic acid per 100 c.c. water neutralised with $CaCO_3$) was added with the acetaldehyde (10 grm. per 100 c.c.); in others acetaldehyde was used alone.

Two cubic centimetres of the above solutions were added to the culture medium each day under sterile conditions, so that each flask received a daily addition of 0·2 grm. of acetaldehyde and the same quantity of formic acid (as formate) in the cases where this was used.

This treatment was continued until in two experiments 60 c.c. of the above solutions had been added, and in two others until 80 c.c. had been added. At the end of this time and occasionally during the progress of the experiment sub-cultures were made to show that the organism in question was still alive and uncontaminated. In two experiments the whole of the acetaldehyde had not been used up, so that the liquid obtained by distillation of the culture medium was strongly reducing to Fehling's solution. In two other cases sufficient time was allowed to elapse between the last addition of aldehyde and the examination of the products to ensure the complete removal of this factor.

In these two cases the distillates obtained had no reducing power. In every experiment the distillate was tested by means of the Voges and Proskauer reaction for acetylmethylcarbinol, and in no case could any trace of this be detected.

The liquid was further examined for butylene glycol, which was in every case found to be present, though in extremely small quantities. It was detected as described above by the formation of diacetyl, this substance being proved to be present in every case by the positive results given by the Voges and Proskauer reaction after oxidation of the culture distillate.

In one case sufficient osazone was prepared from the diacetyl for a determination of the melting point, which was found to be 244° C. Among the products of the reaction were also found ethyl alcohol, acetic acid, and some succinic acid. Lactic acid was not present. These products were estimated in the manner previously described by Harden (13) with a few slight modifications. In the estimation of the alcohol any unchanged acetaldehyde present was removed by oxidation with moist silver oxide. The acetic acid was determined by the method of Macnair (14).

The table below shows the results obtained in three typical experiments:—

Table III.

| | 1. | 2. | 3. |
|---|---|---|---|
| Total acetaldehyde added | 6·07 grm. | 8·0 grm. | 7·6 grm. |
| Total formic acid added (as Ca formate) | 6·07 ,, | Nil | 7·6 ,, |
| | grm. | grm. | grm. |
| Alcohol | 6·5 | 4·02 | 4·5 |
| Acetic acid | 2·5 | 1·38 | 1·3 |
| Succinic acid | Not estimated | 1·25 | 0·12 |
| Butylene glycol | + | 0·677 | 0·109 |

From the point of view of the production of butylene glycol the presence of calcium formate appears to be detrimental, and it is also interesting to note the somewhat large quantity of succinic acid produced in Experiment 2, which also gave the largest yield of glycol.

### *Summary of Results.*

1. *B. lactis aërogenes* and *B. cloacæ*, when grown in a peptone solution containing either glucose, fructose, mannose, galactose, arabinose, isodulcite, mannitol or adonitol, produce both acetylmethylcarbinol and 2.3-butylene glycol.

2. Glycerol, ethylene glycol and acetaldehyde, under similar conditions, also give rise to 2.3-butylene glycol in presence of *B. lactis aërogenes*, but no acetylmethylcarbinol is produced. In these three cases a carbon synthesis is involved, analogous to that which occurs in the butyric fermentation of glycerol and lactic acid.

3. The fermentation of citric and malic acids, of dihydroxyacetone, and of peptone water, gives rise to neither carbinol nor glycol.

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# *The Chemical Action of* Bacillus cloacæ (*Jordan*) *on Glucose and Mannitol.*

By James Thompson.

(Communicated by Arthur Harden, F.R.S. Received November 22, 1911,—Read February 1, 1912.)

(From the Biochemical Department, Lister Institute.)

The close relationship of *B. cloacæ* (Jordan) to *B. lactis aërogenes* (Escherich) suggested the investigation of the chemical action of the former on glucose and mannitol. The two organisms are lactose-fermenting bacilli, allied to *B. coli communis*, and showing a close resemblance to each other in their biological characteristics. *B. lactis aërogenes* is a non-motile, Gram-negative, non-liquefying bacillus, a facultative anaërobe which produces acid and clotting in milk. *B. cloacæ* is a facultative anaërobic bacillus, actively motile, Gram-negative, slowly liquefying gelatine, and producing acid and clot in milk. The chief biological characters of the organisms will be clearly seen in the following table, in which + means acid and gas, — no action:—

| | Glucose. | Lactose. | Cane sugar. | Dulcitol. | Dextrin. | Inulin. |
|---|---|---|---|---|---|---|
| *B. cloacæ* ............ | + | + | + | — | + | — |
| *B. lactis aërogenes* .. | + | + | + | — | + | — |

Harden and Walpole* have already fully investigated the products of the decomposition of glucose and mannitol by *B. lactis aërogenes*, and a comparison of their results with those to be obtained from *B. cloacæ* presented a problem of considerable interest, owing to the fact that both organisms give the Voges and Proskauer reaction. This reaction is due to the presence of acetylmethylcarbinol, which is closely related to butylene glycol, a substance which had been found as one of the products of the fermentation of glucose by *B. lactis aërogenes*. The organism was grown anaërobically in a medium containing 1 per cent. of Witte peptone and 2 per cent. of the sugar in the presence of chalk. The products were examined by the method outlined by Harden† in his investigation of the action of *B. coli communis* on glucose.

An alteration in the method of collecting the evolved gases was made, with the object of eliminating the error involved in collecting over saturated brine, in which carbon dioxide is slightly soluble. The collecting apparatus used consists essentially of an evacuated flask of about 5 litres capacity, and has been fully described in a previous paper.*

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* 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 399, and 1911, B, vol. 83, p. 272.

† 'Chem. Soc. Trans.,' 1901, p. 610.

### A. *Action of* B. cloacæ *on Glucose.*

The substances produced by the action of *B. cloacæ* on glucose were found to be the same as those found in the case of *B. lactis aërogenes*, viz., acetic acid, lactic acid, succinic acid, formic acid, ethyl alcohol, carbon dioxide, and hydrogen. No trace of marsh gas was detected. As in the case of *B. lactis aërogenes* the culture medium at the end of the fermentation gave Voges and Proskauer's reaction, and there was also production of butylene glycol, amounting to 19 per cent. of the sugar. The relative proportions of the products of fermentation differed considerably from those of *B. coli communis* and, in a less degree, from those of *B. lactis aërogenes.* These variations are shown in the following tables. The actual percentages by weight of the products on the glucose fermented in two separate determinations are given in Table I, Columns 1 and 2. For comparison, in Column 3 are given the results of a typical fermentation of glucose by *B. lactis aërogenes,* and in Column 4 those of a similar fermentation by *B. coli communis.* Table II shows the number of carbon atoms per molecule of glucose represented by each product.

On comparing the results given in Table I it will be seen that the ratio of hydrogen to carbon dioxide by volume, viz. 0·3 : 1, in the gas evolved from glucose by *B. cloacæ* is somewhat smaller than in the case of *B. lactis aërogenes* (= 0·42 : 1), and markedly less than for *B. coli communis* (= 1·19 : 1). Theobald Smith† gives the characteristic ratio for *B. lactis aërogenes*

*Glucose.*

Table I.—Percentages.

| | *B. cloacæ.* | | *B. lactis aërogenes.* | *B. coli.* |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| Alcohol | 16·55 | 13·15 | 17·1 | 12·85 |
| Acetic acid | 1·17 | 3·04 | 5·1 | 18·84 |
| Lactic acid | 2·04 | 10·99 | 5·5 | 31·90 |
| Succinic acid | 2·03 | 1·74 | 2·4 | 5·20 |
| Formic acid | 4·31 | 3·20 | 1·0 | 0·0 |
| Carbon dioxide | 41·75 | 41·11 | 38·0 | 18·09 |
| Carbon dioxide, c.c. per grm. | 211·3 | 208·0 | 198·3 | 91·8 |
| Hydrogen, c.c. per grm. | 64·0 | 55·7 | 82·4 | 110·0 |
| Ratio $H_2/CO_2$ | 0·3 | 0·27 | 0·42 | 1·19 |

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* Harden, Thompson, and Young, 'Biochem. Journ.,' 1910, vol. 5, p. 230.

† Theobald Smith, 'Centralb. f. Bacteriol.,' 1895, vol. 18, pp. 1, 494, 589.

*Glucose.*

Table II.—Carbon Atoms.

| | *B. cloacæ.* | | *B. lactis aërogenes.* | *B. coli.* |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| Alcohol | 1·30 | 1·03 | 1·34 | 1·01 |
| Acetic acid | 0·07 | 0·18 | 0·31 | 1·13 |
| Lactic acid | 0·12 | 0·66 | 0·33 | 1·91 |
| Succinic acid | 0·12 | 0·11 | 0·15 | 0·32 |
| Formic acid | 0·17 | 0·13 | 0·04 | 0·00 |
| Carbon dioxide | 1·71 | 1·68 | 1·60 | 0·74 |
| Total | 3·49 | 3·79 | 3·77 | 5·11 |
| Hydrogen, atoms per molecule | 1·04 | 0·90 | 1·33 | 1·77 |

$H_2/CO_2 = 1:1$, but this result does not represent the actual ratio of the gases produced, owing to the solubility of the carbon dioxide in the liquid medium contained in the ordinary fermentation tubes which he employed. This source of error has been obviated, as already pointed out, by collecting the gases over mercury in an evacuated flask. Formic acid was found in the products obtained from *B. cloacæ* in far greater amount than in those from *B. lactis aërogenes,* while those given by *B. coli* are usually almost free from this substance. It is, however, probable that at least a portion of the carbon dioxide is derived from the decomposition of formate primarily formed as an intermediate product. A very marked difference in the relative proportions of alcohol and acetic acid produced by the three organisms will be noticed. While the molecular ratio alcohol/acetic acid for *B. coli communis** is 1, and for *B. lactis aërogenes* (average of three determinations) = 4, that for *B. cloacæ* was found in two experiments to be 18 and 6. The large difference between these results is due to the fact that only a very small amount of acetic acid is produced, and a small absolute difference in this produces a large change in the ratio. Succinic acid is produced by *B. cloacæ* in rather smaller amount than by *B. lactis aërogenes,* and in less than half the quantity given by *B. coli communis.* The amount of alcohol is approximately equal to that given by *B. lactis aërogenes.* A considerable deficiency of carbon in the fermentation of glucose by *B. cloacæ* was found, and, remembering the very similar biological characters of *B. cloacæ* and *B. lactis aërogenes,* butylene glycol was sought for.

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* Harden, 'Journ. Hygiene,' 1905, vol. 5, p. 488.

### *Production of* 2.3-*Butylene Glycol by* B. cloacæ.

A medium containing 50 grm. of glucose in 1 litre of a 1 per cent. solution of Witte's peptone, to which had been added 10 grm. of chalk, was inoculated with the bacillus. After six weeks' incubation at 37°, the liquid was distilled to dryness under reduced pressure at 40°. The dry residue was extracted with boiling absolute alcohol, and the alcoholic solution distilled at 40° under reduced pressure. The residue, weighing 10·6 grm., was fractionated at normal pressure. A fraction distilling between 178° and 184°, weighing 9·5 grm., which solidified completely in a freezing mixture, was obtained. That this substance was 2.3-butylene glycol was proved by converting a portion of it into diacetyl[*] by the action of bromine water under the influence of light. From 4·5 grm. butylene glycol was obtained 1 grm. diacetyl, from which was prepared the phenylosazone.[†] After recrystallisation from alcohol and water the latter was found to have a melting point 243°.

### B. *Action of* B. cloacæ *on Mannitol.*

Considerable differences, while on the whole not so marked as in the case of glucose, are also found on comparing the results of the fermentation of mannitol by *B. cloacæ* with those of *B. coli communis* and *B. lactis aërogenes*. In Table III, Columns 1 and 2, are given the results of two separate determinations of the products resulting from the action of *B. cloacæ* on mannitol, and in Columns 3 and 4 are given for comparison the figures obtained by typical fermentations of mannitol by *B. lactis*

*Mannitol.*

Table III.—Percentages.

| | *B. cloacæ.* | | *B. lactis aërogenes.* | *B. coli.* |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| Alcohol | 27·45 | 26·48 | 32·5 | 28·1 |
| Acetic acid | 4·23 | 3·67 | 2·5 | 9·5 |
| Lactic acid | 2·64 | 2·24 | 8·6 | 18·6 |
| Succinic acid | 2·29 | 4·24 | 3·2 | 8·9 |
| Formic acid | 5·56 | 4·56 | 1·5 | 3·0 |
| Carbon dioxide | 29·02 | 31·20 | 35·5 | 28·44 |
| Carbon dioxide, c.c. per grm. | 146·8 | 157·8 | 180·3 | 143·0 |
| Hydrogen, c.c. per grm. | 110·2 | 116·9 | 138·3 | 167·0 |
| Ratio $H_2/CO_2$ | 0·75 | 0·74 | 0·77 | 1·18 |

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[*] v. Pechmann, 'Ber.,' 1890, vol. 23, p. 2427.

[†] v. Pechmann, 'Ber.,' 1888, vol. 21, p. 2754.

### *Mannitol.*

Table IV.—Carbon Atoms per Molecule of Mannitol.

| | *B. cloacæ.* | | *B. lactis aërogenes.* | *B. coli.* |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| Alcohol | 2·17 | 2·10 | 2·57 | 2·22 |
| Acetic acid | 0·26 | 0·22 | 0·15 | 0·58 |
| Lactic acid | 0·16 | 0·14 | 0·52 | 1·13 |
| Succinic acid | 0·14 | 0·26 | 0·20 | 0·55 |
| Formic acid | 0·22 | 0·18 | 0·06 | 0·12 |
| Carbon dioxide | 1·20 | 1·29 | 1·47 | 1·16 |
| Total | 4·15 | 4·19 | 4·97 | 5·76 |
| Hydrogen, atoms per molecule | 1·80 | 1·91 | 2·26 | 2·7 |

*aërogenes* and *B. coli communis* respectively. Table IV shows the number of carbon atoms per molecule of mannitol represented by the various products. As in the case of glucose, a considerable deficiency in the carbon will be noticed; this is to be accounted for by the production of acetylmethylcarbinol and butylene glycol, a qualitative experiment having shown the presence of both these substances.

In the case of mannitol there is practically no difference in the ratios $H_2/CO_2$ for the two organisms *B. cloacæ* and *B. lactis aërogenes.* As with glucose, the amount of formic acid obtained from *B. cloacæ* is considerably greater than from either *B. lactis aërogenes* or *B. coli communis.* On the other hand, the opposite is to be observed in the figures given for succinic acid. A comparison of Tables I and III shows that *B. cloacæ* produces twice as much alcohol from mannitol as from glucose. This further confirms the suggestion previously made by Harden* that the formation of alcohol in these reactions is related to the presence of the terminal $CH_2(OH)\cdot CH(OH)$ group, which occurs twice in the molecule of mannitol, and only once in that of glucose.

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* Harden, 'Chem. Soc. Trans.,' 1901, p. 610.

# Herpetomonas pediculi, *nov. spec., Parasitic in the Alimentary Tract of* Pediculus vestimenti, *the Human Body Louse.*

By H. B. FANTHAM, D.Sc., B.A., Christ's College, Cambridge, and Liverpool School of Tropical Medicine.

(Communicated by Prof. Sir Ronald Ross, K.C.B., F.R.S. Received November 24, 1911,—Read January 18, 1912.)

[PLATE 14.]

## INTRODUCTION.

The organism which forms the subject of this paper is a small flagellate Protozoön occurring in the alimentary tract of the body louse, *Pediculus vestimenti* (*P. corporis*). The flagellate belongs to the genus Herpetomonas, and is, I believe, now recorded for the first time. I propose for it the name *Herpetomonas pediculi,* using the name Herpetomonas in the sense of Saville-Kent (1881), the founder of the genus.

The parasite was first seen by me nearly three years ago, when working in Cambridge. At that time, material being scanty and apparently difficult to obtain, there seemed to be no special reason for recording the presence of yet another species of Herpetomonas, though the possibility of lice acting as carriers of disease was realised. Other researches were then in progress, but I have given *H. pediculi* intermittent attention ever since I discovered it. Lately it has been suggested that flagellates belonging to the genera Herpetomonas and Crithidia occurring in the digestive tracts of blood-sucking insects are really stages in the life-history of vertebrate trypanosomes. In consequence of the importance of the subject in relation to the transmission of trypanosomes and of the increasing attention devoted to leishmaniasis (or human herpetomoniasis), I have resumed my study of *Herpetomonas pediculi,* and have found its complete life-cycle.

I hope to show that, although *Herpetomonas pediculi* might easily be confused with dangerous parasites transferable to man by the bite of the insect hosts, yet it is really a harmless flagellate of the gut of the louse. Bred lice, fed on my own blood, have been used in the research.

## MATERIAL AND METHODS.

The hosts of *Herpetomonas pediculi* were common body lice, *Pediculus vestimenti.* The lice were originally obtained from the bodies of infested children and from verminous clothing recently removed from the body.

Some of the lice so obtained were dissected, and found to contain Herpetomonads. The adult lice were isolated into glass tubes, containing small fragments of white woollen material. They clung to the material, and excrement from them adhered to it. Examination of the said excrement in a very few cases showed non-flagellate stages of the parasite. Lice, thus known to be infected, were then paired, as also were lice that were apparently uninfected. The parent lice were kept in closed tubes in contact with my body, and were fed twice daily, either on my arm or on the back of my hand. They fed greedily, often evacuating fæces as they fed, and at times even unaltered fresh blood. The eggs from these lice were kept also in tubes, and, when the larvæ hatched, they were treated in the same way as the adults.

Detailed examination was made of the eggs, larvæ, and adult lice, and it was found that bred lice from parents known to be infected were parasitised by *H. pediculi.* Though clean lice bred clean, the young ones became infected when they came in contact with the fæces of infected lice.

Again, fæces known to be infected were smeared on spots at which uninfected young lice were allowed to feed. Three days later, control insects showed no flagellates or other form of the parasite, while those fed at infected spots contained active flagellates when dissected.

The sole food supply of the bred lice was my own blood. Cultures of my blood on Mathis' modification of Novy and MacNeal's medium have never yielded any flagellates of any sort.

The percentage of infected lice is not a high one—I have dissected as many as 50 lice at a time without finding Herpetomonads—nor can the infection be described as heavy ; 300 Pediculi were examined, of which 25 were found to be infected. Much time has been devoted to the examination of fresh material, which is absolutely essential if the life-cycle is to be followed with exactitude. The gut of the louse was divided into small serial portions, each of which was finely teased and examined in physiological salt solution. Observation of the living organisms was supplemented by examination of thin smears of the gut contents, teased intestinal wall, and other organs, and long search was made for possible intracellular stages.

Wet fixation with osmic vapour, followed by absolute alcohol, was used, and coloration with Giemsa's or other modification of the Romanowsky stain. Very dilute solution of methylene blue was of use as an *intra vitam* stain.

It may be noted that lice dissected when several hours had elapsed since the last feed showed the parasites better than recently fed ones. The presence of much blood in the gut interferes with the study of the living organism, as well as adds to the difficulty of staining.

## OCCURRENCE OF THE PARASITES IN THE HOST.

*Herpetomonas pediculi* exhibits three typical stages in its life-history—the pre-flagellate, flagellate, and post-flagellate stages. So far the examination of the mouth parts of the lice has shown no parasites. The pre-flagellate stage of the parasite occurs chiefly in the œsophagus and proventriculus of the adult louse, and throughout the alimentary canal of the immature insect or larva. The flagellate stage is found at its best in the mid-gut of the mature louse, while the extreme intestinal and rectal portions of the gut contain the post-flagellates. The latter are also found in the excrement. The late larva may contain a few flagellate parasites.

Detailed examination was made of the reproductive organs and ova of the lice, but no parasites were found therein. The possibility of the hereditary infection of the lice with *H. pediculi* was, in my opinion, excluded so far as the specimens that I examined were concerned. The parasites have not been found in organs other than those of the alimentary tract. The diverticula of the alimentary canal apparently are uninfected.

## MOVEMENTS.

Movements of *H. pediculi* are most easily observed in the fully developed flagellate stage. They are also very vigorous during division, but as the object of the said movements then is to complete the fission of the organism, they are of a somewhat exceptional character.

Usually movement is brought about by waves of contraction followed by relaxations passing down the body, while progression is aided by the lashing of the flagellum, which is forwardly directed. The type of movement is somewhat euglenoid, and the occasional concentration of the cytoplasm into the anterior end produces a "peg-top" effect that is very characteristic. The progression is somewhat spasmodic, consisting of alternate slow movements and rapid darts forward, accompanied by a slight rolling from side to side. Reversal of the direction of motion is easily accomplished. The organism either swings as a whole in a semicircle, the posterior end acting as a centre of rotation, or the flagellum bends back parallel to the body, which then swings suddenly in a semicircle, and so comes to lie in a straight line with the flagellum, after which the parasite moves away in the opposite direction from what it was traversing previously.

The flagellum often lashes vigorously, and when obstacles are encountered appears to test them by touching them rapidly in different spots.

Rotatory movements occur when the Herpetomonas becomes fixed to *debris* in the lumen of the gut. The organism lashes its flagellum violently, and the flagellum describes a series of circles and spirals, producing marked currents in its neighbourhood.

A very common movement is that of partial rotation of the body of *H. pediculi.* The posterior end of the body remote from the nucleus is chiefly concerned, and this portion often twists over, so that the dorsal surface becomes ventral and *vice versâ* (Plate 14, fig. 10). Sometimes a small cluster of parasites may be observed, twisting simultaneously and producing a general shimmering effect.

Entanglement of two organisms by their flagella is sometimes seen. The movements of the parasites then are most violent. On one occasion I saw the flagellum of a small parasite torn from its body by the vigorous movements of a much larger Herpetomonas with which it had become entangled. Such intensely vigorous movements as the last-mentioned are rare.

## Life-History in Brief of *H. pediculi.*

It is of interest to trace the course of the development of the parasite throughout its life in one host. The following development has been observed in the living organism, and afterwards corroborated by examination of stained preparations.

In the excrement of lice infected with *H. pediculi* are small, oval bodies, well adapted for resisting desiccation or other adverse condition. Similar bodies occur in the hind gut of the lice, where they are formed before passing out with the excrement. Other lice, feeding at spots contaminated by their predecessors, ingest some of these small oval post-flagellates, which consist of a "varnish-like" thin cyst wall, enclosing some granular protoplasm, a nucleus and a blepharoplast (Plate 14, figs. 27–29). Such cysts may also be ingested by larval Pediculi. Passing with fresh blood into the fore gut of a new host, the post-flagellates commence a new development, and as this leads to the formation of the flagellate, it has been termed the pre-flagellate stage (fig. 1).

At first round or oval, the parasite rapidly commences to elongate, the end first lengthening becoming, as a rule, the flagellar end of the organism. At this period, a somewhat more refractile area can be seen in life, and the finely granular chromatophile contents of this area concentrate, forming a thread which ultimately reaches the surface. The thin ectoplasm of the parasite is pushed forward still more by the thread, which ultimately becomes free of the body and protrudes as a short flagellum (fig. 2). The flagellar origin is the chromatophile area or so-called "flagellar sac," which is usually in the neighbourhood of the blepharoplast, which, in the fully developed organism, is always anterior to the nucleus. Growth of the non-flagellar end is continuous, and by the time that the flagellum has reached its full length the non-flagellar (or posterior) end has elongated and become fully developed.

During the actual flagellate stage (figs. 8–18) little in the way of actual development occurs, but the vital activities of the organism are displayed by vigorous multiplication. Increase in numbers in *H. pediculi* takes place by longitudinal division. The parasites about to divide seem to grow broader just prior to the act and to become more granular. The first indication of division is shown by the concentration of the substance of the blepharoplast into two masses which are connected together by a very narrow neck. The dumb-bell like body so formed (fig. 19) gradually separates into two distinct blepharoplasts, placed slightly obliquely, one on either side of the body (fig. 20). The intra-cellular part of the flagellum (or rhizoplast) commences to divide just after the blepharoplast, and the split appears to extend forwards. Concentration of the nuclear material occurs simultaneously, and the nucleus becomes constricted, usually in the median line and parallel to the long axis of the body, but occasionally markedly to one side. The constriction deepens, and ultimately two nuclei are produced. These migrate to the sides of the organism, and fission of the general cytoplasm commences (figs. 20, 21). The flagella lash about very much at this time, and their vigorous action aids in the separation of the daughter organisms. The latter gradually diverge until they come to lie in a straight line, and finally become separated from one another at the apex. The fission is practically always followed by active swimming movements of the daughter organisms. Consequent on this great activity of the daughter forms immediately after division, rosettes of Herpetomonads, due to repeated longitudinal division and non-separation of the resultant parasites, are exceptional. Division is best seen in the mid-gut of the louse.

After a series of longitudinal divisions, resulting in the production of a number of flagellate individuals, a reaction sets in, and the parasite prepares for life outside the body of its host. As the Herpetomonad passes backwards into the very dark semi-digested blood in the hind gut of the louse, the chromatin of its flagellum dwindles, and appears to be absorbed (figs. 22–25). The cytoplasm concentrates around the nucleus, to which the blepharoplast also is drawn nearer. The parasite becomes more or less rounded or oval, and proceeds to secrete a thin, gelatinous wall, which rapidly hardens to a "skin-tight" coat around the organism, which, in this sense, may be said to encyst (figs. 26–28). Thus prepared and protected, the oval bodies, now known as post-flagellates, pass from the gut of the host, mingled with the dejecta to recommence the life-cycle if ingested by a new host.

## MORPHOLOGY.

### A. *The Pre-flagellate Stage.*

The pre-flagellate stage (figs. 1–4) of *H. pediculi,* at its earliest, takes the form of small oval or rounded bodies, measuring 6 $\mu$ to 7 $\mu$ by 4 $\mu$ to 5 $\mu$. They have a marked resemblance to the Leishman-Donovan bodies. The pre-flagellate shows a thin ectoplasm and endoplasm containing refractile granules. The nucleus (fig. 1) is oval or occasionally rounded, while the deeply staining blepharoplast (kinetic nucleus) may be bar-like, oval, or occasionally rounded, and is well marked (figs. 1, 2). The position of the blepharoplast varies somewhat, as would be expected in a developing organism. It may lie to the side of the nucleus or above it, and occasionally the blepharoplast is apposed to the nucleus. The chromatophile area (fig. 1) from which the flagellum differentiates is also present. Division occurs in the pre-flagellate stage (fig. 5), more especially when the organism is beginning to elongate, and possesses a short flagellum. Isolated chromatoid granules are sometimes present in the pre-flagellates (fig. 2). The appearance of intermediate forms (figs. 2–4, 6, 7) between the pre-flagellate and the flagellate has been indicated in the section dealing with the life-history.

### B. *The Flagellate Stage.*

The flagellate form (figs. 8–18) of *H. pediculi* is an active organism relatively small compared with other Herpetomonads, its body length varying from 11 $\mu$ to 26 $\mu$ in the specimens examined. The inclusion of the flagellum doubles the length of the organism, for the flagellum itself may be 30 $\mu$ in length (fig. 16). The cytoplasm of the organism is finely alveolar (figs. 8–18), and very refractile in life. Chromatoid granules (figs. 15, 18) are present in some cases. The protoplasm rarely presents marked vacuoles in stained preparations, but in life a clearer area is sometimes seen near the origin of the flagellum.

The nucleus (figs. 8–18) is round or oval, and crowded with very fine granules. A karyosome is seen in some cases (figs. 11, 15) but is not visible in all, doubtless being masked by the numerous fine granulations present. A nuclear membrane apparently occurs, but is not so chromatic as when the nucleus is of a vesicular type. The blepharoplast (kinetic nucleus) may be oval or rod-like, lying transversely across the body, or somewhat obliquely. Occasionally it is curved, and it often presents a bowed appearance prior to division. It stains deeply, taking a purplish tint with Giemsa. Before the onset of the multiplicative phase the blepharoplast presents no differentiation. The free flagellum tapers finely at its free end. It originates as a rhizoplast near the blepharoplast, and occasionally a minute basal granule can be distinguished with difficulty.

*Aggregation rosettes.*—Just as division rosettes are infrequent, so aggregation rosettes or clusters are uncommon. It is noteworthy that the members of an aggregation rosette (fig. 13) may be of different sizes and ages. In such rosettes, the parasites either mass themselves around some food particle with which they are in contact by their flagella, or else several organisms intertwine their flagella and so form a sort of bouquet or ball of living organisms, all vibrating slowly from a common centre provided by their interlaced flagella. These rosettes in time break up into the component units. One after another, the slow-moving organisms manage to detach themselves and swim away until the last two separate. The object underlying these simple rosette formations is not fully understood. Possibly it enables the flagellates to withstand better any currents in the gut, and so gives them a somewhat longer lease of life as flagellates, before encystment overtakes them.

### C. *The Post-flagellate Stage.*

The post-flagellate stages of *H. pediculi* (figs. 25–29) when fully formed are small bodies (less than the pre-flagellates) containing protoplasm and a nucleus, to which the blepharoplast may be apposed, or in which nucleus and blepharoplast can be distinguished as separate entities. The blepharoplast is often somewhat smaller than in the other phases of the parasite. The cyst wall is extremely thin, staining pinkish after Giemsa (figs. 27, 28). The blepharoplast is not always easy to demonstrate in stained preparations, but that it must be present is obvious when one has taken the trouble to watch the process of post-flagellate formation in the living animal and has studied a series of stained preparations of intermediate forms (figs. 22–26). Cysts with thick, radially striated walls (fig. 29) have very rarely been encountered, and I am inclined to think that the presence of the swollen gelatinous wall containing striations and enclosing a parasite with chromatoid granules is a sign of degeneration.

## *Herpetomonas pediculi* IS A NATURAL PARASITE OF *Pediculus vestimenti.*

Recently much controversy has arisen from statements made to the effect that flagellates found in sanguivorous insects must be regarded as developmental stages of trypanosomes. Accordingly—ignoring the evidence of life-cycle and morphology—*H. pediculi* would be regarded by some as a phase of a trypanosome. Sweeping statements such as that quoted are rarely logical, and when they are based upon a series of speculations and single instances instead of on an accumulation of facts, they are usually unsound.

While it is quite true that certain trypanosomes, *e.g.*, *T. lewisi*, assume a Herpetomonas-like form in cultures, yet they are not then under exactly natural conditions. Further, they may be considered as reverting to the type from which it seems probable that they have originated, namely, primitive Herpetomonads which have undergone morphological changes and in process of time have evolved the trypanosome type when inoculated into the vertebrate host.

With regard to *H. pediculi*, I do not think that there is any doubt that it is a flagellate, natural to and parasitic in the insect host, and that it has no connection with a human trypanosome, pathogenic or non-pathogenic. In support of this conclusion, I cite the following facts and experiments:—

(1) At various times during the past three years, I have fed lice on my blood from the time of hatching until they died. A tsetse fly transmitting *Trypanosoma gambiense* is at first limited in its period of infective inoculation. Lice might also be similarly limited, but, owing to the method of feeding adopted, no question of the lice not having fed at their infective period can be entertained. In spite of repeated feedings of lice, my blood shows no sign of trypanosomes, whether tested by ordinary microscopical examination of films, by thick films or by cultural methods, and the period covered by the experiments is ample to have allowed of full development of trypanosomes, were *H. pediculi* a phase of one.

(2) Artificially infected lice have been fed simultaneously. The result of mass feeding surely should have been sufficient to produce some indication of trypanosomes, were any present. No such indications have been found, even after inoculation of my blood into susceptible animals like white rats. (Animals examined for six weeks after inoculation.)

(3) The experiment of inoculating rats with the contents of the gut of lice containing *H. pediculi* has given no positive results whatever. The rodents remained perfectly healthy, nor did cultures of their blood, or thick film examinations, yield any trace of trypanosomes.

(4) Cultures of the gut contents of infected lice showed no further stage in the life-history of the parasite.

(5) The methods of infecting larvæ and adults of *P. vestimenti* with *H. pediculi* have been briefly indicated in a preceding section. The same contaminative method of infection has been observed under natural conditions, and resembles that found in the case of some other insects, such as *Pulex irritans* (adult and larva), infected with *Crithidia pulicis* (Porter, 1911) and *Nepa cinerea*, harbouring *Herpetomonas jaculum* (Léger, 1902; Porter, 1909).

Further, the well-defined development of *H. pediculi*, with its pre-flagellate, flagellate and post-flagellate forms, presents a cycle complete in itself, and there is no evidence to show that there is any connection with the life-cycle of any other organism.

Contamination of experimental *P. vestimenti* by feeding on other vertebrates has been rigorously excluded, so that no fallacious results can accrue from outside sources.

From the foregoing considerations, the conclusion obviously must be that if *H. pediculi* be a stage in the life-history of a vertebrate trypanosome, the said trypanosome should most probably be present in my blood, and should have revealed itself by now. Repeated cultures, thick-film blood examinations, and ordinary smears, examined continuously during this research, have all proved negative. Hence, all the evidence available points to the fact that *H. pediculi* is a parasite of the insect *Pediculus vestimenti,* and has no connection with any trypanosome of persons on whom lice may feed. Were such a trypanosome to exist, it is surprising that it has not been recorded ere this, considering the number of blood examinations undertaken in various scientific institutions.

Further, I do not think that *H. pediculi* has any connection with *Leishmania,* as no symptoms of leishmaniasis have developed in me, and England is a country free from the disease. However, the possible occurrence of such a natural *Herpetomonas* in lice must be remembered in experimenting with Pediculi as possible transmitters of *Leishmania.*

"Wild" lice—the term commonly used to denote lice that were not bred for purposes of investigation but collected at random—obtained from several widely different districts in England, have also yielded the flagellates when dissected. Doubtless, were more lice available from other areas, some also would be infected. The inference is then, I think, fairly justified that *H. pediculi* occurs in a few body lice throughout England.

Some Continental authorities would perhaps place *H. pediculi* in the genus *Leptomonas.* However, I have followed most English workers in considering that members of the genus *Herpetomonas* are really uniflagellate, as originally defined.

### NOTE ON THE BIOLOGY AND LIFE-HISTORY OF *Pediculus vestimenti.*

The study of parasitic Protozoa demands a good knowledge of the life-history and habits of the host. In dealing with lice, great difficulty was at first experienced, as the literature on the subject is very scattered and unsatisfactory. Since the commencement of this research, a valuable paper by Warburton (1909) has appeared, which gives details as to the length of life of the lice, time of incubation, and rearing of the larvæ. I can fully confirm all that Warburton has recorded.

The eggs of *P. vestimenti* vary in their incubation period. I found that while a few eggs hatched in four to five days, others matured as much as six weeks after laying. Warburton found the same kind of variation. The larvæ were pale coloured, and fed as soon as they left the egg, if placed on the back of the hand. Moulting occurred every four days, the new skins being slightly darker than the previous ones. The larvæ fed very greedily, and were much more active than the adults. When feeding, a larva has sucked blood for as long as 25 minutes, peristaltic waves being clearly visible in the gut the while. Usually 10 to 15 minutes' feed was sufficient.

The imaginal stage is attained about eleven to twelve days after hatching, sexual maturity about four days later. Copulation is intermittent, but frequent. In several cases it occurred shortly after feeding, particularly when the insects fed greedily, so that unchanged fresh blood occasionally passed from their bodies after the semi-solid digested blood *débris* had ceased to be voided. Egg laying at the rate of four or five per day occurs during the rest of the life of the female, who is longer-lived than the male. Warburton found that the adult life of a male was about three weeks, that of a female four weeks. In my own experiments similar results were obtained, but I also found that the length of life was sometimes about a week less, in each sex.

The mode of feeding of adult *P. vestimenti* is of interest. After settling down on the hand, often clinging to the scrap of cloth on which they usually rest, a fairly sharp stab is made, and immediately the blood begins to flow into the alimentary canal, which becomes bright red. As feeding proceeds, the louse gradually raises its abdomen, until it is almost vertical in extreme cases. As fresh blood passes into the gut, defæcation occurs, much excrement being produced. If an attempt be made to remove a louse before it has finished its feed, the pull of the ring of hooks near the lower lip can be felt. Lice fed in a somewhat restricted area showed no hesitation in sucking blood at spots fouled by themselves or their neighbours. Adult lice would feed for 20 to 30 minutes. If feeding were neglected, the lice died in about three days. I found it necessary to feed them at least twice daily, though I have succeeded in keeping two females alive for three weeks when fed only once a day. Larvæ perish if not fed within 36 hours of hatching, and even then there is great loss during the larval stage.

Lice are also very sensitive to changes of temperature. Body heat seems necessary for them, though eggs can withstand great extremes of temperature.

Death of *P. vestimenti* appears to occur very suddenly. I found that a fair number of those adults that died did so within a short time after a meal, their alimentary canals containing much unchanged blood.

Regarding the specific name of the body-louse there is much uncertainty. Neumann, in a recent paper (July, 1911), suggests that *P. vestimenti* is a sub-species of *P. capitis*, and would then be called *P. capitis vestimenti.* However, a discussion of such a difficult matter of nomenclature is quite outside the scope of this paper.

### SUMMARY AND CONCLUSIONS.

1. *Herpetomonas pediculi* is a parasite of the body louse, *Pediculus vestimenti.* The parasite appears to be confined to the alimentary tract and fæces of its host (adult and larva), one phase of it having been recovered from the dejecta. The parasite is spread from louse to louse by the contaminative method, cysts of the parasite being swallowed by the insect. The whole life-cycle has been followed in the living material.

2. Movements of the flagellate are very rapid and somewhat spasmodic, and are easily accomplished by the aid of the flagellum. Rotatory motion and movements of flexion occur.

3. The parasite exhibits three well-marked developmental phases, united by a continuous series of intermediate forms:—(i) The pre-flagellate, which produces a flagellum and elongates (figs. 1—7), and becomes (ii) the flagellate (figs. 8—18), which, after a growing and multiplicative phase by longitudinal fission (figs. 19—21), forms (iii) the resting, "encysted" post-flagellate form, adapted for extra-corporeal life (figs. 22—29).

4. Pre-flagellate stages, best found in the œsophagus and proventriculus of the louse, or in the larva, strongly resemble Leishman-Donovan bodies. They are $6\,\mu$ to $7\,\mu$ long and $4\,\mu$ to $5\,\mu$ broad. The nucleus and blepharoplast are well defined. A chromatophile area, from which the flagellum develops, is present.

5. The flagellate forms occur chiefly in the mid-gut. The body length is from $11\,\mu$ to $26\,\mu$ in those I have examined. The cytoplasm is finely alveolar. The nucleus is round or oval, and the blepharoplast stains deeply. Aggregation rosettes of flagellates of various ages and sizes are occasionally found (fig. 13).

6. Post-flagellate forms are oval, usually provided with a "skin-tight" cyst. The blepharoplast seems smaller than that of the flagellate or pre-flagellate. This stage is best observed in the rectum of the louse and can be recovered from the fæces.

Radially striated, thick-walled cysts occur very rarely (fig. 29).

7. My experiments show that *H. pediculi* is not a stage of a vertebrate

trypanosome, for I have fed the infected lice from the time of hatching to the time of death on my own body, and have made detailed examinations of my own blood by smears, thick films, and by cultures, as well as by subinoculations into white rats, none of which has ever given indication of trypanosomes during three years of experiments. Animals inoculated with *H. pediculi* from the gut of lice have also shown no parasites.

8. *H. pediculi* is a parasite of the louse, *Pediculus vestimenti,* and shows no connection with any vertebrate trypanosome. Also, it is not connected with *Leishmania.*

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## EXPLANATION OF PLATE 14.

All figures outlined with Abbé-Zeiss camera lucida, after wet fixation with osmic vapour and absolute alcohol and staining with Giemsa's solution; 2 mm. apochromatic objective (Zeiss) and compensating oculars 8 and 12 used. Magnification 1500 diameters.

Figs. 1—7 represent pre-flagellate and intermediate forms of *H. pediculi* from the digestive tract of the larva or the fore-gut (œsophagus and proventriculus or anterior lobe of the stomach) of the adult *Pediculus vestimenti.*

In fig. 1 note the chromatophile or pink-staining finely granular area from which the flagellum arises.

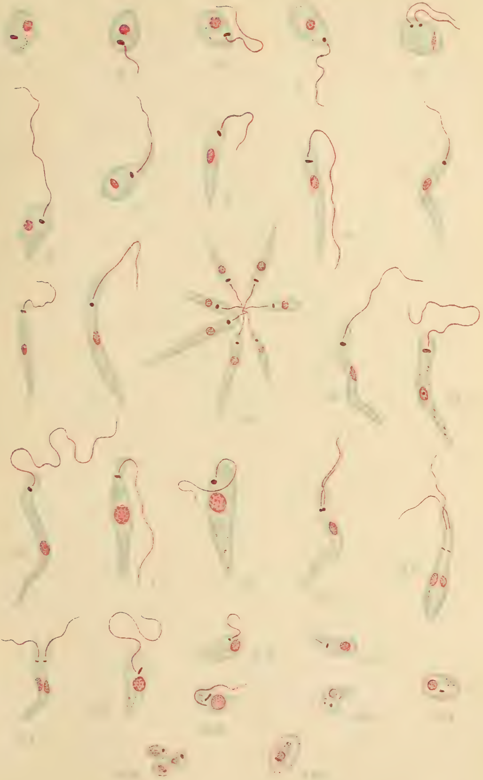
In figs. 2, 3, 4, 6, 7 note the gradual lengthening of the flagellum and elongation of the body.

Fig. 5 represents a late pre-flagellate organism dividing.

Figs. 8—18 show flagellate parasites from the stomach and anterior part of the intestine (or mid-gut) of the adult louse.

Fig. 8. Young flagellate.

Fig. 10. Flagellate showing body twisted or folded over about the middle of its length.



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HERPETOMONAS PEDICULI.

Fig. 13 represents an aggregation rosette or cluster of flagellates of different sizes and ages.

Figs. 14, 15, 16 show parasites whose bodies are thrown into characteristic undulations. Note the chromatoid granules shown in fig. 15.

Figs. 17, 18 represent stout flagellates from the anterior part of the intestine. The latter figure shows the stoutest parasite seen during the research. The parasite contained chromatoid granules.

Figs. 19—21 represent dividing forms.

Figs. 22—25 show stages of the parasite leading to post-flagellates and cysts (figs. 26—29), as seen in the hinder part of the intestine, including the rectum and the fæces.

In figs. 22—25 note the gradual shortening and absorption of the flagellum and the contraction and rounding of the body. In fig. 25 only the rhizoplastic part of the flagellum remains. Chromatoid granules occur in these parasites.

Figs. 27—29 represent truly encysted forms, as found at the extreme posterior end of the gut or voided in the fæces with semi-solid, black blood remains.

In figs. 27, 28 the cyst-wall is thin and varnish-like, and closely apposed to the parasite. It stains pink after Giemsa.

Fig. 29 represents a gelatinous, thick-walled cyst with striations. Such cysts were very rare.

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# *A Method for Isolating and Cultivating the* Mycobacterium enteritidis chronicæ pseudotuberculosæ bovis, *Jöhne, and Some Experiments on the Preparation of a Diagnostic Vaccine for Pseudo-tuberculous Enteritis of Bovines.*

By F. W. Twort, M.R.C.S., L.R.C.P. Lond., and G. L. Y. Ingram, M.R.C.V.S.

(Communicated by Leonard Hill, M.B., F.R.S. Received November 7, 1911,—Read February 1, 1912.)

(From the Laboratories of the Brown Institution, University of London.)

The disease of cattle described under the name of pseudo-tuberculous enteritis, or Jöhne's disease, is a serious affection which causes considerable losses to farmers and stockowners throughout the British Isles and Europe. Clinically, it is characterised by a slow progressive emaciation and chronic diarrhœa, which causes the milk-yield in cows to fall off, and often ends in death. B. Bang states that some cases show no diarrhœa, though the affected animals die; he also states that the annual losses from this disease on some of the large farms in the Islands of Denmark may amount to 5 to 8 per cent. of the total head of cattle. The disease may affect bovines of all ages (usually from 3 to 6 years, Meissner (33)), but as the period of incubation has been shown to be very long, it is not usually recognised in animals which are less than a year old. It often appears in pregnant cows, and becomes aggravated after calving.

On *post-mortem* examination the lesions are found to be confined to the bowels and the surrounding lymphatic glands. The mucous membrane of the small intestine is seen to be very much thickened—in some cases it may be three or four times the normal. It is thrown into characteristic folds or corrugations, and may show areas of congestion. The large intestine and cæcum often present the same lesions. The affected glands are enlarged and œdematous, but show no caseation.

Scrapings taken from the thickened mucous membrane, and stained by Ziehl-Nielsen's method, as a rule show enormous numbers of acid-fast bacilli, though in some cases they are less numerous, and in others may be very difficult to discover.

In sections of an affected intestine the bacilli are found to be most numerous near the surface, *i.e.* towards the lumen of the bowel, but they are also found in the villi and in the deeper layers. The increase in thickness is seen to be due to the formation of new connective tissue. "The tissue is filled with large epithelioid cells, surrounded by small round lymphatic cells, and in some cases with giant cells" (B. Bang).

The presence of an acid-fast bacillus, not to be distinguished microscopically from the tubercle bacillus, in the thickened mucosa of the bowels of cattle suffering from chronic diarrhœa, was first shown in 1895 by Jöhne and Frothingham (1), who considered the condition to be a form of tuberculosis, and with them Koch was in agreement.

In 1881 J. Hansen and P. H. Nielsen, of Holland, pointed out the thickening and corrugation of the mucous membranes of the intestines of certain cattle dying from chronic diarrhœa, whilst Hurtrel d'Arboval, in 1826 ('Dict. de Med. et de Chirurg. Vét.'), under the head of chronic enteritis in cattle, described conditions which might well have been due to the micro-organism now known as Jöhne's bacillus. Bouley and Reynal do not seem to have recognised it as a special form of enteritis ('Dict. de Chirurg., Méd., et d'Hygien Vét.,' 1860).

The disease is prevalent in many countries. Van der Sluys (3) and Markus (5) have described its occurrence in Holland, Liénaux and van den Eeckhout (7) in Belgium, B. Bang (14) in Denmark, and Borgeaud (8) in Lausanne.

Jöhne described the first case in Dresden, and Bongert (12), Meissner (33), and others have reported many further cases in Germany. Fréger (13), Matthis (15), and Lechlainche (18) in France, and Horne (24) in Norway, have also recognised and conducted experimental work on this disease.

In North America, the first case was described by Pearson in Pennsylvania, in 1908, and since then it has been recognised by Beebe (25), of Minnesota, and others.

In 1906 B. Bang (37), of Copenhagen, demonstrated the disease, and showed microscopic preparations of the diseased gut and glands before the National Veterinary Association at Liverpool; he suggested that many cases of chronic diarrhœa ascribed to various intestinal strongyles were really due to Jöhne's bacillus, and predicted that the disease would be found in Great Britain, as he had in his own experience found it in tubercle-free cattle imported from Jersey.

In 1907 McFadyean (19) described cases in this country occurring among Shorthorn, Sussex, and Jersey cattle, and, later, observed one case in a deer. In 1909 Stockman, whilst investigating a disease of sheep in Scotland, known locally as "Scrapy" or "Scrapie," found acid-fast bacilli in lesions corresponding to those of Jöhne's disease. Previous to this, in 1907, Liénaux attempted to inoculate the disease in sheep, but was apparently unsuccessful.

In 1909, in the Report of the United States Bureau of Animal Industry, mention is made by Dr. J. Mohler of an attempt to cultivate the bacillus of Jöhne's disease from specimens received from California, but it is clear from the details given that the specific bacillus was not grown.

Many attempts have been made to produce this disease experimentally in cattle and in the usual laboratory animals. As far as can be ascertained, in no animal, except cattle, has inoculation proved successful. Meissner and Trapp (34) state that they have only been able to produce the disease in calves. This they have done, as also has B. Bang, by intravenous and intraperitoneal inoculation of large quantities of infected material, and also by feeding calves with considerable quantities (1 to 3 lbs.) of the mucous membranes of the intestines of cattle dying from this disease. Experiments of a similar nature on mice, guinea-pigs, rabbits, sheep, and goats have all proved negative. In many instances the results have caused confusion, since the material used has been taken from animals also suffering from tuberculosis, and this strengthened the theory that the condition was a form of tuberculosis affecting the bowels and surrounding lymphatic glands, but showing no tendency to caseation. B. Bang, by feeding a calf with 300 grm. of affected material, found that the animal showed signs of diarrhœa in eight months, and this evidence of the long period of incubation has been supported by other observers.

All writers on this disease state that the causative micro-organism cannot be cultivated outside the animal body. Meissner (33), however, obtained on a decoction of grass (*Phleum pratense*) and glycerine agar a pure culture of an acid-fast bacillus which Koch and Rabinowitsch (17) declared to be identical with the bacillus of avian tubercle. Mettam also, in a private communication, states that he has sub-cultured for 12 generations an acid-fast micro-organism obtained from a cow suffering from Jöhne's disease, and that it agrees in every respect with the avian tubercle bacillus. These must be regarded as cases of accidental contamination with the avian tubercle bacillus.

Most authors agree that animals suffering from this disease give no reaction with ordinary diagnostic tuberculin, but O. Bang (38) has obtained in some cases a more or less decided reaction with avian tuberculin. In this country Male (39), of Reading, has since used avian tuberculin prepared by Stockman. He tested 19 cattle, giving 5 c.c. doses of tuberculin, and in four cases obtained a reaction of 3·6°, 3°, 4°, and 2·8° F. respectively. He does not state, however, whether the pre-inoculation temperature given is that of the morning or evening, and it must be remembered that both Meissner and Mettam have obtained avian tubercle bacilli from the intestine of cows infected with Jöhne's bacillus.

In June, 1910, we started some experiments with the object of cultivating Jöhne's bacillus and of preparing a diagnostic vaccine from the culture obtained. A preliminary note on the results of this work was included in a paper by one of us (41) on the cultivation of the lepra bacillus of man, published in 1910.

We have to thank Mr. Brennan de Vine, of Birmingham, and Mr. D. Hamilton, of Hamilton, for the pathological specimens used in these experiments.

Portions of infected intestine and glands were obtained in as fresh a condition as possible, but owing to the time taken in transit, some of the specimens were found to be contaminated in the deeper tissues, and it was found necessary to kill off these contaminations with a solution of ericolin—a method which was originally devised for isolating tubercle bacilli (42). The gut and glands were thoroughly washed in water, and the surface of an infected area seared with a hot spatula; microscopic films were made from the tissues beneath to prove the presence of the specific bacillus. Small pieces of tissue were then removed with sterile scissors and rubbed over the culture medium to be tested, either directly, or indirectly after being incubated in a 1 per cent. watery solution of ericolin for one to two hours at 37° C.

The first case received from Mr. de Vine, on June 15, 1910, showed the typical lesions of pseudo-tuberculous enteritis, and contained a large number of acid-fast bacilli. The specimen was fresh, and cultures were made directly on to all the ordinary laboratory media. Fresh extracts of various glands and organs (including the intestine) of normal bovines were also prepared, and sterilised by passing through a Doulton white filter. The extracts thus sterilised were placed in sterile tubes plugged with cotton wool, and inoculated with the diseased material. Small sterile portions of bovine organs were also obtained, placed in sterile tubes, and inoculated. The above media were tested in various combinations, both with and without glycerin, cholesterin, various sugars, fresh blood, and other substances. The cultures were made aërobically and anaërobically at 39° to 40° C. On none of these media were we able to obtain any definite growth of the specific bacillus.

Some experiments were also conducted to test the possibility of an ultra-microscopic virus working in symbiosis with Jöhne's bacillus. Extracts of bovine intestine infected with the disease were prepared and passed through a Doulton white filter. The sterile filtrate so obtained was added to the various media, and the whole inoculated with portions of intestine containing living Jöhne's bacilli. These experiments all gave negative results.

From the experiments conducted on this case we came to the conclusion arrived at by most other workers, namely, that the specific bacillus would not grow on any artificial medium known to bacteriologists, and that if successful cultivation were to be achieved some new medium would have to be prepared. We considered also that the failure of growth of the specific bacillus must be due, either to some substance in the medium acting as a poison, or to the absence of some material or foodstuff necessary for its vitality and growth.

On considering the question further, we were struck by the apparent close relationship existing between this micro-organism and the tubercle bacillus; and since the bacillus of pseudo-tuberculous enteritis and the tubercle bacillus both grow in the same species of animal, we considered it highly improbable that there could be any substance in the ordinary laboratory media which would act as a poison to the one bacillus and not to the other. This possibility was accordingly excluded, and we were forced to conclude that the failure to grow the bacillus must be due to the absence of some necessary foodstuff.

Considering again the apparent close relationship between the tubercle bacillus and the bacillus of pseudo-tuberculous enteritis, and the fact that both these bacilli live in the bodies of bovines, we judged it probable that they would require the same chemical substances for building up their protoplasm, certain of which substances could be elaborated from artificial media by the tubercle bacillus, but not by the bacillus of pseudo-tuberculous enteritis—in other words, that the latter bacillus has lived a pathogenic existence from such remote ages, that it has lost the original power of its wild ancestor—whatever bacillus that may have been—and can no longer build up all its necessary foodstuffs outside the animal body.

It was thought probable that if these substances could be obtained ready formed and added to some good artificial medium (Dorset's egg medium), the bacillus would grow, and further, that these substances might be elaborated by allied micro-organisms, such as the tubercle bacillus, and even stored up as reserves in their envelopes. On this reasoning, which led to the successful cultivation of the lepra bacillus of man (41), we decided to prepare media containing these allied bacilli which had been killed by heat.

We had at the time in our possession about three hundred strains of tubercle bacilli, mostly isolated from human tuberculous material on Dorset's egg medium. A number of these cultures were taken, and after the necessary sub-cultures had been made, they were killed by steam. The growth was then scraped off, taking care to avoid any admixture of the medium which might contain the waste products of the bacillary growth and be toxic to the bacillus of pseudo-tuberculous enteritis. More recently we have found this precaution to be unnecessary. The growth of tubercle bacilli thus obtained was ground in a mortar with glycerine and saline, steamed for half an hour, and added to the yolk and white of new laid eggs in the following proportions: Egg 75 parts, 0·8 sodium chloride in re-distilled water, 25 parts; these were thoroughly mixed, and to the mixture were added tubercle bacilli 1 per cent. and glycerine 5 per cent. This medium was placed in sterile test-tubes, these were plugged with cotton wool and heated in a hot water bath at 60° C. for one hour on three successive days, the tubes being incubated at 37° C. for 6 to 12 hours in the intervals between steaming. Finally the tubes were inspissated in slopes at 85° to 90° C.

A second case of pseudo-tuberculous enteritis was now obtained from Mr. de Vine. Specimens of intestine and glands were received on July 28, 1910. Both the intestine and glands showed the typical characters of the condition, and a large number of Jöhne's bacilli were present in various parts of the tissues. Unfortunately, owing to the hot weather prevailing at the time, the specimens on delivery had commenced to decompose, but, in spite of this, we prepared some cultures in the manner previously described, both directly, and indirectly after treating with ericolin solution. The cultures were made on several of the media tested with the first case, as well as on a number of tubes of the special tubercle bacillus medium. The tubes were capped with gutta-percha tissue and incubated at 39° to 40° C. After two days' incubation all the direct cultures were badly contaminated, yet those inoculated with ericolinised material showed only a few contaminating colonies. Sub-cultures were made from uncontaminated areas of most of the latter tubes on to fresh tubes of the same medium, but, owing to the small amount of the tubercle bacillus medium then prepared, only one of these tubes was sub-cultured —this was one made from a gland. Films from these sub-cultures were prepared at intervals of about four or five days, and examined microscopically. After 19 days the sub-culture on the special medium showed quite definite evidence of multiplication, the bacilli had grown larger and thicker, they were well stained, and were present in large close masses. Sub-cultures were made from this tube on fresh tubes of various media, including one tube of the special tubercle bacillus medium. These were examined at intervals as before, and the sub-culture on the special medium showed microscopic evidence of growth in 10 days. Both the first and second sub-cultures showed growth visible to the naked eye after four weeks, which gradually increased, and reached a maximum in about eight weeks.

These tubes were easily sub-cultured on to fresh tubes of the same medium, but on none of the ordinary laboratory media were we able to get any evidence of growth.

The third case of pseudo-tuberculous enteritis was obtained from Mr. Hamilton. Specimens of intestine, but no glands, were received on September 23, 1910. They showed the typical lesions of the disease, and a very large number of Jöhne's bacilli were present in the tissues. When delivered, the specimens had already commenced to decompose, but from them cultures were made as previously described, both directly, and indirectly after treatment with ericolin solution, on various media, including some tubes of the tubercle bacillus medium. The tubes were capped with gutta-percha tissue and placed in an incubator at 39° to 40° C. The results were the same as in Case No. 2; all the direct cultures were badly contaminated, and those tubes which had been inoculated with material previously treated with ericolin solution grew only a few contaminating colonies. Of the latter, the cultures on the tubes of special medium were sub-cultured from uncontaminated areas on to a number of fresh tubes of various media, including the special medium. The sub-cultures on the ordinary media remained sterile, but those on the tubercle bacillus medium grew Jöhne's bacillus in pure growth, and were, without difficulty, repeatedly sub-cultured on to fresh tubes of the special medium. Naked eye evidence of growth was present in the first sub-cultures after about six weeks.

The fourth case was obtained from Mr. de Vine, a specimen of intestine being received at the Institution on January 26, 1911. It showed the typical lesions of pseudo-tuberculous enteritis, and a large number of the specific bacilli were present in the lesions. Since the specimen was quite fresh, cultures were made as previously described from the ileum, cæcum, and ileo-cæcal valve directly on to nine tubes of the special tubercle bacillus medium; these were capped and placed at 39° to 40° C. After three weeks' incubation two tubes were found to be contaminated, whilst the remainder were covered with extremely minute colonies of Jöhne's bacillus without any contaminations; the cultures grew well, and were sub-cultured without any difficulty on to the special medium. Sub-cultures taken on to Dorset's egg medium, glycerine agar, and various other media, gave no growth.

Case 5 was obtained from Mr. Hamilton, and was received at the Institution on February 8, 1911. The specimen, consisting of ileum and ileo-cæcal valve, showed the typical lesions of pseudo-tuberculous enteritis, and a considerable number of acid-fast bacilli were present in the lesions. Cultures were made from several parts of the specimen directly on to 12 tubes of Dorset's egg medium. They were taken in the manner already described, but as the specimen was fresh on arrival, previous treatment with ericolin solution was unnecessary. The tubes were capped with gutta-percha tissue, and placed in the incubator at 39° to 40° C. On the following day they were examined and found to be free from contaminating colonies, so the tiny pieces of tissue were removed from three of the tubes and placed on to three tubes of the special tubercle bacillus medium. These were capped and placed with the other tubes in the incubator at 39° to 40° C. Six weeks later the three tubes of special medium showed a few tiny colonies of Jöhne's bacillus. Compared with the previous cases the rapidity of growth was very slow and was slight in amount, due, as was proved later, to the unsuitability of the particular strain of tubercle bacillus incorporated in the medium. Sub-cultures from these tubes on to tubes of a fresh batch of tubercle bacillus medium grew well. All the original cultures on Dorset's egg medium remained sterile, as also did sub-cultures from the special medium on to Dorset's egg medium.

Four strains of Jöhne's bacillus having been isolated on media containing dead tubercle bacilli, we next proceeded to test them on slightly modified media. We found that growth was not nearly so good in the absence of glycerine, but the exact percentage most suitable for the growth of Jöhne's bacillus has not yet been determined, although we have reason to believe that about 4 per cent. by volume gives the best results. A higher per cent.

sodium chloride solution can be used in preparing the medium, without any detrimental effect.

We found also that it was better to dry the growth of the tubercle bacillus after killing it and before making it up into medium, a fact which may be due to the formation of cracks or breaks in the continuity of the covering, enabling the essential substance to diffuse more easily into the medium. Experiments showed that ½ to 1 per cent. of the dried tubercle bacillus was the most suitable quantity to add. To obtain the best results, the dried bacilli should be ground up with the glycerine which has been mixed with an equal quantity of 0·8 per cent. saline, and the remainder of the saline added later. The emulsion so obtained should then be steamed for 15 minutes, and, when cool, added to the egg. The probable explanation for this is that the glycerine acts as a solvent for the essential substance, and some experiments to be described later tend to confirm this suggestion. We also tried some media similar to the above, in which the normal alkalinity of the egg was wholly or partially neutralised by hydrochloric acid; these were found to be unsuitable. This proves the necessity of maintaining a distinctly alkaline reaction.

In another series of experiments the egg was replaced by various other substances, such as broth or agar. These, as a rule, did not give such good results, although ordinary glycerine peptone bouillon, made distinctly alkaline, and containing ½ to 1 per cent. of dried tubercle bacilli, gave a fairly satisfactory growth. This, with other experiments to be described later, proved that Jöhne's bacillus can grow quite well in the absence of albumen.

We next proceeded to test our strains of Jöhne's bacilli on media in which the dead tubercle bacillus was replaced by various other micro-organisms. We soon found that some strains of human tubercle bacilli were more suitable than others; and, further, that if the human tubercle bacillus was replaced by the bovine type, no growth of Jöhne's bacillus took place, and that this was so even when sub-cultured from strains which had been growing outside the animal body for a year. Several strains of tubercle bacilli isolated from cats were also tested, but gave negative results.

The question then arose as to whether these results were due to the absence of some substance in the bovine tubercle bacillus, or to the presence of some toxic substance not found in the human type. This was tested by preparing four batches of medium, one containing ½ per cent. of dried human tubercle bacilli, another ½ per cent. of dried bovine bacilli, a third ½ per cent. of both the human and bovine types, and a fourth ¼ per cent. of both types. Several tubes of each were inoculated with pure cultures of

Jöhne's bacillus and incubated at 39° to 40° C., with the following results:—Growth took place on the medium containing the human type and on the two containing both types, but no growth took place on that containing only the bovine type. This experiment proves fairly conclusively that the unsuitability of the bovine type of bacillus is not due to the presence of any toxic body in its substance, otherwise no growth would have taken place on the media containing the mixture of the two bacilli. We may note, however, that we have not tested many strains of the bovine bacillus, and it is possible that Jöhne's bacillus will grow on some bovine strains, or on those strains which have been described as occupying an intermediate position between the typical human and typical bovine bacilli; but we have no evidence that this is so.

Whatever this difference between the two types of bacilli may be due to, it does not in our opinion necessarily represent an important biological difference; it is probably physiological in nature, and may be due to the presence or absence of some reserve food material existing or otherwise outside the strictly vital portion of the bacillus, or it may be due to some fat, wax, or other covering material preventing this substance from being utilised by Jöhne's bacillus. In the light of some recent experiments the latter possibility seems improbable, as we have been unable to extract any substance suitable for the growth of Jöhne's bacillus. These experiments are being continued.

While in this paper we cannot enter into the controversy concerning the relationship between the human and bovine types of tubercle bacilli, yet, incidentally, we venture to remark that, in spite of all that has been written in this country, we are not yet convinced that the human and bovine types are only slightly different varieties of one and the same micro-organism. In this connection the difference between the two bacilli described above may be worthy of note and further investigation.

The failure to obtain any growth of Jöhne's bacillus on media containing tubercle bacilli isolated from bovines and cats led us to seek for other acid-fast bacilli which might act as substitutes for the tubercle bacillus of man, and two bacilli at once suggested themselves. As we have already remarked, O. Bang has shown that avian tuberculin may cause some reaction with pseudo-tuberculous enteritis of bovines, and the possibility of the avian tubercle bacillus and Jöhne's bacillus being closely allied is at once obvious.

Accordingly we prepared several batches of medium containing the avian tubercle bacillus in place of the human type. On this medium our strains of Jöhne's bacillus usually grew, but only slightly, and the medium proved to be quite unsuitable for practical purposes.

The other micro-organism which suggested itself was the timothy-grass bacillus. From very remote times this bacillus must have been repeatedly ingested by bovines in their food, and it seems quite possible that it may be the wild ancestor and originator of Jöhne's bacillus which now infests the intestine, causing pseudo-tuberculous enteritis. Batches of medium containing ½ per cent. of this bacillus in place of the human tubercle bacillus were prepared, placed into tubes, and sterilised in the manner previously described. A number of these tubes were inoculated with pure cultures of Jöhne's bacillus and incubated at 39° to 40° C. as before. These cultures grew quickly and well, the growth being better than on any of the media containing the human tubercle bacillus. A slight growth was visible along the needle track after incubation for one week, and after six weeks the growth closely resembled that of a bovine tubercle bacillus recently isolated from the animal body. A full description of the cultural characters of the bacillus will be given later. The smegma bacillus of Moeller, the nasenschleim bacillus of Karlinski, and the fish tubercle bacillus of Dubard were then tested in place of the human tubercle bacillus; each type was added to the medium in quantities of ½ to 1 per cent. of the dried powdered growth. The first two media gave satisfactory results, but were not quite so good as media containing the timothy-grass bacillus. The fish tubercle bacillus medium gave negative results, but so far only one batch of this has been tested.

The butter bacillus of Rabinowitsch was also found to be unsuitable. Certain blastomyces and non-acid-fast bacilli which have been recently investigated also gave negative results.*

In the above experiments it must be noted that we were testing the various media with vigorous growing strains of Jöhne's bacillus, some strains of which had been growing outside the animal body for nearly a year. The question now arose as to whether we should have obtained the same good results with such micro-organisms as the timothy-grass bacillus, had we started by inoculating the media directly with bovine tissue infected with Jöhne's bacillus, instead of with cultures which had been growing outside the animal body for a considerable period.

To test this point Mr. de Vine kindly sent us a further specimen of diseased gut, and this, our sixth case, was received at the Institution on July 28, 1911. The specimen was delivered in a fresh condition, and showed the appearance of pseudo-tuberculous enteritis, most marked near the ileo-cæcal valve; the disease was in an early stage, and the thickening of the intestine was quite moderate. Films were made from the ileum and the ileo-cæcal valve, but only a very few Jöhne's bacilli could be found, even after searching for some considerable time. Small pieces of tissue were removed aseptically in the manner previously described and inoculated on to six tubes of Dorset's egg medium, and on to two tubes of special medium containing ½ per cent. of dried timothy-grass bacillus. All were capped with gutta-percha tissue and incubated at 39° to 40° C. as with the previous cases.

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* Subsequent experiments have shown that the following acid-fast bacilli can also be used in the medium, and give good results :—*B. Pseudoperlsucht*, Moeller ; *B. aus Harn*, Marpmann ; *B. aus Butter*, Grassberger. No positive results have yet been obtained with the Tobler group of acid-fast bacilli (January 29, 1912).

After 48 hours one of the tubes of special medium was found to be contaminated and was discarded, After five weeks' incubation films were made from the tubes and examined microscopically. Those taken from the cultures on Dorset's egg medium showed no acid-fast bacilli, but that taken from the remaining tube of special medium, made with dead timothy-grass bacilli, showed some small clumps of acid-fast bacilli presenting the characters of Jöhne's bacillus. Accordingly sub-cultures were made from this tube on to fresh tubes of the same medium and on to fresh tubes of Dorset's egg medium. All the tubes were capped and placed in the incubator at 39° to 40° C. Films were now made at intervals of about a week from the various tubes, and without describing all in detail it will be sufficient to note that the bacillus found on the original tube of special medium continued to grow on this and on all the sub-cultures made on to the timothy-grass bacillus medium, and on media containing the human tubercle bacillus, but that the sub-cultures on Dorset's egg medium remained sterile. The bacillus isolated resembled in every way the bacilli isolated from the four previous cases, and the cultural characters were also the same.

From the above experiments it is clear that Jöhne's bacillus will grow on media containing the dead timothy-grass bacillus, not only after it has been cultivated in the laboratory for a considerable period, but also when taken direct from the diseased gut of cattle.

Having determined the various acid-fast bacilli most suitable for the growth of Jöhne's bacillus, an attempt was made to extract the essential substance from certain of these bacilli. The timothy-grass bacillus was chosen, chiefly because it gave the best results in the above experiments, also because it is harmless to man and grows quickly on simple media, thus enabling a large quantity of growth to be obtained in a short time.

Dr. W. Bulloch kindly gave us a quantity of this bacillus, besides various dead and dried tubercle bacilli, which latter had been given to him by Prof. Bang about eight years previously. Many of these had already been extracted by Bulloch and MacLeod (40) when investigating the acid-fast properties of the tubercle bacillus. The different bacillary powders were made up into media, the tubercle bacillus of our original medium being replaced by one or another in quantities of $\frac{1}{2}$ per cent. Tubes of each were inoculated with a fresh culture of Jöhne's bacillus, and the results may be summarised as follows:—

Dried timothy-grass bacilli gave very good results.
„ human tubercle bacilli „ good results, but inferior to the timothy grass bacillus.
„ bovine „ „ negative results.
„ swine „ „ „
„ tubercle of uncertain source, freed from wax and fat, gave negative results.
„ „ „ „ freed from wax, fat, and proteid, gave negative results.

The dried timothy grass bacillus and the dried human tubercle bacillus were found to be equally good when previously autoclaved in normal saline for 30 minutes at 120° C. The above results prove conclusively that the essential substance contained in these bacilli is comparatively stable, remaining undiminished in timothy grass and human tubercle bacilli which had been dried and killed eight years previously, and also after they had been autoclaved.

Some further experiments were now made: 1 grm. of dried timothy grass bacilli was taken and extracted with 20 c.c. of 0·8-per-cent. sodium chloride and 4 c.c. of glycerine. The mixture was autoclaved for half an hour at 120° C. and passed through filter paper. The filtrate was then added to the white and yolk of hens' eggs in the proportion of one part of filtrate to three parts of egg. Another batch of medium was prepared by taking the residue of the timothy-grass bacillus, washing it repeatedly with normal saline, filtering it and drying the residue. This residue was made up into medium, the tubercle bacillus of the original tubercle egg medium being replaced by $\frac{1}{2}$ per cent. of the residue of the timothy-grass bacillus. Further batches of medium were prepared by extracting the dried timothy-grass bacillus with distilled water, the necessary quantities of sodium chloride and glycerine being added after extraction and filtration. The residue was treated as before.

We found that Jöhne's bacillus grew on the medium containing the glycerine saline extract, and on that containing the residue. It also grew on the residue after extraction with distilled water, but it failed to grow on the medium containing the distilled water extract. From these results we judge that the essential substance is only very slightly, if at all, extracted by distilled water, but that it is soluble in a glycerine saline solution, although from the above it is clear that some of the essential substance remained in the residue.

A further series of experiments was made, using ethyl alcohol as our solvent. Two grammes of dry timothy grass bacillus were powdered, placed in a Soxhlet apparatus with 100 c.c. of absolute alcohol, and extracted for three hours. The residue was dried in an incubator, and the alcohol evaporated to dryness, leaving a dark yellowish sticky mass. The extract and residue were then weighed separately, and it was found that the original weight of the bacilli was reduced from 2 grm. to about 1·25 grm., the difference being represented by the extract. Media were prepared with the extract and residue thus obtained, the tubercle bacillus of our original medium being replaced by 1 per cent. of the extract or residue. Other batches of these media were thus prepared, some of which contained only ¼ or ½ per cent. of the extract or residue. Tubes from each batch were inoculated from young growths of Jöhne's bacillus, and incubated at 39° to 40° C.

Good growth was obtained on all the media containing the extracts, but, as a rule, there was none on the residues.

These experiments prove that the substance in the timothy-grass bacillus essential for the growth of Jöhne's bacillus is extracted by hot ethyl alcohol. As is well known, if this hot alcoholic extract is allowed to cool, a white flocculent precipitate forms, and can be removed by filtration. The clear coloured filtrate, when evaporated to dryness, leaves a thick oily residue which becomes firmer on cooling. Part of this residue is soluble in hot and cold chloroform, leaving an insoluble liquid portion which floats on the surface of the chloroform, but is soluble in water.

Media prepared with any one of these different parts of the alcoholic extract give positive results with Jöhne's bacillus, the best being that which is insoluble in chloroform.

So far these extracts have not been purified, and it is possible that the essential substance contained in each portion is identical.

In considering pseudo-tuberculous enteritis from an hygienic standpoint some important factors have to be considered. The disease is widely distributed, and is easily conveyed from one animal to another, probably by means of contaminated food, such as grass. In the early stages the symptoms are slight and indefinite, and an early diagnosis is impossible. The affected animals lose considerable weight, and with milch cows the quantity of milk given is greatly diminished. In view of these facts it is clear that a reliable diagnostic vaccine would be of great economic value, and if generally used in the infected areas of the countries in which the disease is prevalent, and if followed by the slaughter of the diseased animals, would soon tend to diminish if not to completely eradicate this condition.

It must also be remembered that when animals suffering from this disease are slaughtered, the flesh is not condemned as food unless the carcase shows marked emaciation, so that an early diagnosis would not only help to prevent dissemination of the disease, but would allow of a better price being obtained for the animal.

From this it follows that the use of such a vaccine would be of direct monetary value to farmers and stockowners, and if State legislation were adopted, no Government compensation would be necessary.

From the strains of Jöhne's bacillus which had been grown, we now attempted to prepare a vaccine which would be both efficient and specific as a diagnostic agent for the disease under discussion. In the first experiments we used an alkaline peptone bouillon, containing 4 per cent. of glycerine and 1 per cent. of dried human tubercle bacilli. This was placed in Duclaux flasks and sterilised by steaming. These flasks were inoculated with pure cultures of Jöhne's bacillus, and the main opening of each was capped with gutta-percha tissue. The flasks were incubated at 39° to 40° C. After the lapse of a month small yellowish-white grains of growth became visible. These grew just above the sediment at the bottom of the flasks, and gradually increased in size and number. No film formation was observed. After two months the flasks were steamed, their contents passed through a Doulton white filter, and the filtrate so obtained placed into small sterile flasks in quantities of 2½ and 5 c.c. The vaccine was not evaporated to obtain a more concentrated solution, as we considered this unnecessary for experimental work.

A second batch of vaccine was prepared in a manner exactly similar to the above, except that the dried human tubercle bacillus was replaced by the dried timothy-grass bacillus. A third batch was prepared by growing Jöhne's bacillus in a broth medium containing a glycerine saline extract of the timothy-grass bacillus, so as to represent 1 per cent. of dried bacilli and 4 per cent. of glycerine. The medium used for preparing the last vaccine was filtered and autoclaved before inoculating, and in it the specific bacillus grew fairly well as tiny masses which settled down to the bottom of the flasks. Incidentally, this proved that Jöhne's bacillus will grow in an albumen-free medium.

A fourth batch was prepared from cultures of Jöhne's bacillus on the special timothy-grass bacillus egg medium, the growth being scraped off and an emulsion made with vaccine No. 3 described above.

A fifth batch of vaccine was made in a manner similar to vaccine No. 4, except that the three cultures of Jöhne's bacillus were suspended in 6 c.c. of 0·8-per-cent. sodium chloride in place of vaccine No. 3.

As controls to the above vaccines we used diagnostic tuberculin prepared by the Pasteur Institute, diagnostic avian tuberculin prepared at the Royal Veterinary College, and a special timothy-grass bacillus vaccine prepared by ourselves. This last was made in the same manner as ordinary diagnostic tuberculin. The bacillus was grown for about three weeks in a glycerine broth medium, which was then steamed and filtered through a Doulton white porcelain filter. The sterile filtrate was placed in small sterile flasks without previous concentration. The results of the tests conducted with the above vaccines will be described under the head of experiments on bovines.

At the time that test-tube experiments were being carried out we performed a number of inoculation experiments on animals. In the first series we tested small laboratory animals such as mice, rats, guinea-pigs, rabbits, hens, and pigeons. Several of each were inoculated either subcutaneously or intraperitoneally, and some, such as rabbits and pigeons, intravenously, while others were fed with food soaked in an emulsion of the bacilli. Vigorous growing cultures of Jöhne's bacillus were made up into thick emulsions with sterile normal saline; the mice and rats were usually inoculated with $\frac{1}{4}$ c.c., and the guinea-pigs, rabbits, hens, and pigeons with $\frac{1}{2}$ to 1 c.c. of the emulsion.

With the mice, rats, guinea-pigs, hens, and pigeons the results were entirely negative. No lesions were found in the animals *post mortem*, even when kept for nine months before killing.

One mouse inoculated into the peritoneal cavity and killed after 14 days showed a few acid-fast bacilli in one of the mesenteric glands, but they could not be recovered in cultures, and it is probable that they were dead and had been taken up by the glands. Sections of the mesenteric glands showed no histological lesions.

The only rabbit which showed any evidence of a lesion, probably produced as a result of the inoculation (into the peritoneal cavity with 1 c.c. of a thick emulsion of Jöhne's bacillus), was killed after four months. On *post-mortem* examination a small whitish thickening was found in the wall of the cæcum, and films made from this area showed a few degenerated acid-fast bacilli. Sections showed destruction and degeneration of the tissues comprising the wall of the gut, with granular structureless material containing a few degenerated acid-fast bacilli in the centre of the area. Cultures made from this area on to various media, including the special medium containing the human tubercle bacillus, all remained sterile. It is probable that the lesion observed was caused by the inoculation of a large quantity of Jöhne's bacillus into the actual substance of the gut, the needle of the syringe piercing this when the inoculation was made, and so producing an immediate necrosis of the surrounding tissues before the bacilli were killed by the animal fluids and tissues, or during the process. We consider the above experiment to be negative.

Six bovines were also inoculated with pure cultures of Jöhne's bacillus, and it is to be regretted that circumstances did not allow us to perform a larger number of inoculations. We also regret that lack of space prohibits us from describing each in detail. The bare results are given in the accompanying table (p. 534).

Of the experiments, Nos. 3 and 6 need little comment. No. 3 was killed after a lapse of only six weeks, and No. 6 died after 17 days. In both cases the time was too short for the disease to develop, and in neither of the cases were any of the vaccines tested. Accordingly no conclusions can be drawn from either experiment.

Of the four remaining bovine experiments, Nos. 1 and 5 developed pseudotuberculous enteritis, but also showed some tubercular lesions. Nos. 2 and 4 showed no evidence of Jöhne's disease on *post-mortem* examination, although No. 2 showed some tubercular lesions.

In the two bovines which developed Jöhne's disease the lesions, although definite, were not advanced, and only very few bacilli were found, even in the first bovine, which had been fed 11 months previously. These two experiments, taken together with the two negative results, indicate the slow progress of the disease, and, compared with tubercle, the low pathogenicity of Jöhne's bacillus. But it must be remembered that the experimental animals were fed on hay, bran, oats, and mangolds, as we had no opportunity of turning them out to grass, a procedure which is known to greatly accelerate the progress of the disease. It must also be remembered that the bacilli may have become less virulent by growing on artificial media, but we believe the diet to be the more important factor in determining the susceptibility to the disease and its rapidity of progress after contraction. We succeeded in recovering Jöhne's bacillus from the diseased intestine of bovine No. 5, and the cultures obtained were identical in every respect with the culture inoculated.

The coincident tuberculosis in the two positive cases probably played some part in lessening the resistance to Jöhne's bacillus.

The various vaccines which were tested on the experimental animals were also tested on three uninoculated young calves, and on a pedigree bull proved

| Bov. No. | Date inoculated. | How given. | 27/6/11. | 27/7/11. | 28/8/11. | 6/9/11. | 12/10/11. | 18/10/11. | Killed. | P.M. *re* Tubercle. | P.M. *re* Jöhne. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 19/11/10 | Mouth | Vacc. 1.<br>5 c.c.<br>+ | Tuberculin.<br>3½ c.c.<br>+ | Vacc. B.<br>Phleum.<br>8 c.c.<br>– | Av.<br>tuberculin.<br>8 c.c.<br>– | | Vacc. 5.<br>3 c.c.<br>– | 23/10/11 | + | + |
| 2 | 10/11/10 | Intra-ven. | Vacc. 1.<br>2½ c.c.<br>+ | Tuberculin.<br>2 c.c.<br>+ | Vacc. B.<br>Phleum.<br>5 c.c.<br>– | Av.<br>tuberculin.<br>5 c.c.<br>– | | Vacc. 5.<br>2½ c.c.<br>– | 24/10/11 | + | – |
| 4 | 21/2/11 | Intra-perit. | Vacc. 1.<br>2½ c.c.<br>? + ? – | Tuberculin.<br>2 c.c.<br>– | Vacc. B.<br>Phleum.<br>5 c.c.<br>– | Av.<br>tuberculin.<br>5 c.c.<br>– | Vacc. 4.<br>8 c.c.<br>– | | 16/10/11 | – | – |
| 5 | 21/2/11 | Intra-ven. | Vacc. 1.<br>2½ c.c.<br>+ | | | | | | 12/7/11 | + | + |
| Control calf, uninoculated, No. 1 | | | Vacc. 1.<br>2½ c.c.<br>– | Tuberculin.<br>2 c.c.<br>+ | Vacc. B.<br>Phleum.<br>5 c.c.<br>– | Av.<br>tuberculin.<br>5 c.c.<br>– | | | | | |
| No. 2 | | | | Tuberculin.<br>2 c.c.<br>– | Vacc. B.<br>Phleum.<br>5 c.c.<br>– | Av.<br>tuberculin.<br>5 c.c.<br>– | | | | | |
| No. 3 | | | | Tuberculin.<br>2 c.c.<br>– | Vacc. B.<br>Phleum.<br>5 c.c.<br>– | Av.<br>tuberculin.<br>5 c.c.<br>– | Vacc. 4.<br>5 c.c.<br>– | | | | |
| Bull, Jöhne's disease, naturally contracted | | | 1/9/11<br>Vacc. 1.<br>10 c.c.<br>– | | | 1/11/11.<br>Av.<br>tuberculin.<br>8 c.c.<br>– | Vacc. 4.<br>10 c.c.<br>– | | | | |

to be free from tuberculosis but with well developed pseudo-tuberculous enteritis. The bull was kindly presented to us by a breeder interested in this disease, and showed marked clinical symptoms, besides numerous clumps of acid-fast bacilli in the fæces.

The vaccine inoculations and results are set forth in the accompanying table, a + sign indicating a positive reaction and a − sign a negative one. On referring to the table it will be noticed that vaccine No. 1 gave a positive result with bovines Nos. 1, 2, 4, and 5, but not with the control bull. It is also evident from the tuberculin tests and from *post-mortem* examination that animals 1, 2, and 5 had contracted tuberculosis.

In considering these results it must be remembered that vaccine No. 1 was prepared by growing the specific bacillus on a medium containing the tubercle bacillus, and it might be expected that tubercular animals would react to the vaccine on account of the substances dissolved from the tubercle bacilli in the medium, in which case the positive results obtained do not prove the presence of pseudo-tuberculous enteritis. That the rises of temperature with vaccine No. 1 were actually caused by the presence of these substances is finally proved by the absence of any reaction in the control bull, and also by the negative results obtained with vaccines Nos. 4 and 5, which contained no tubercle bacilli.

From the results obtained with vaccine No. 1 it is quite clear that if a specific vaccine for pseudo-tuberculous enteritis is to be obtained, the tubercle bacillus must not be incorporated in the medium. It will be seen from the table that a timothy-grass bacillus vaccine causes no rise of temperature in normal animals, or in animals suffering from tuberculosis or pseudo-tuberculous enteritis. This bacillus should therefore be suitable for incorporating in the medium.

It will be remembered that vaccines 4 and 5 were prepared by growing Jöhne's bacillus in media containing the timothy-grass bacillus; but, as will be seen from the table, these vaccines caused no reaction in any of the experimental or control animals, which further proves the harmlessness of the timothy-grass bacillus.

It may seem surprising that no reaction was obtained with these vaccines in the animals affected with Jöhne's disease, but, in point of fact, the negative results might have been expected, as the greater part of the bacillary emulsion used was obtained from growths on solid media. It must also be remembered that infected animals rarely, if ever, show a temperature during the course of the disease, and it is probable that a more concentrated vaccine will be required for Jöhne's disease than for tuberculosis.

We are now conducting experiments with this object. Up till recently we have been unable to obtain any growth of Jöhne's bacillus on the surface of fluid media, and this has been the chief difficulty in preparing an efficient vaccine, but we have now succeeded in inducing it to grow in film formation on glycerine broth containing the timothy-grass bacillus. We consider this a great advance, as the bacillus should now grow more vigorously, as is usually the case with other acid-fast bacilli.

The specific bacillus of pseudo-tuberculous enteritis, commonly known as Jöhne's bacillus, is, as already stated, allied to the various tubercle bacilli, and therefore belongs to the same general group. If we accept the classification of micro-organisms adopted by Lehmann and Neuman, it would be more correct to describe it as a mycobacterium, and the scientific name of the micro-organism would then be *Mycobacterium enteritidis chronicæ pseudotuberculosæ bovis* Jöhne, the name by which we suggest it should be known. At the same time, throughout this paper we have thought it better to retain the common name of Jöhne's bacillus. We were unable to detect any difference in the five strains isolated, and the culture recovered from the experimental bovine No. 4 also agreed in every detail with the other bacilli. In the diseased lesions the bacilli often appear in extremely large numbers. They are present as slender rods, sometimes slightly bent, and are usually between 1 and 2 microns in length. They often show a beaded appearance, but this is not so marked as with the tubercle bacillus. When first cultivated from the animal body on the special media described in this paper, the bacilli grow longer and thicker, and lie side by side in a manner very similar to the tubercle bacillus. If the medium is partially dried they may grow to a length of 5 microns or more, and show definite dichotomous branching, with club formation and very distinct beading. When sub-cultured on to moist solid medium, the bacilli soon regain their slender and short form, and in vigorous growing cultures they may become very short and show but little beading. The bacilli at this stage often lie side by side, but loosely and not in the typical manner of the early cultures. Spore formation was not observed in any of our growths.

In none of the strains have we been able to detect any evidence of motility.

Like the tubercle bacillus, Jöhne's bacillus, if obtained from diseased tissues or from a pure culture, stains imperfectly and with difficulty with aqueous solutions of the anilin dyes, but quite well with Gram's method, and better still with Ziehl-Nielsen's. It is quite acid-fast, and retains the stain when treated with 25-per-cent. sulphuric acid or 1-per-cent. hydrochloric acid in spirit.

No growth was observed in the absence of oxygen or with considerable excess of oxygen. If sub-cultures which have been kept anaërobically for three months at 39° C. are placed under aërobic conditions, the cultures grow, showing that although the bacillus does not grow anaërobically it is not killed by the absence of oxygen.

Growth occurs between 28° C. and 43° C., or perhaps a little beyond these limits, the optimum being about 39° C., but it is always slow. The reaction of the medium should be distinctly alkaline; the degree of alkalinity possessed by new laid eggs is very suitable, and if this is in any way lessened in the medium there is a marked diminution in the rapidity and amount of the growth. As has been stated, no growth occurs on any of the artificial media in general bacteriological use, such as peptone bouillon, agar, gelatine, serum, potato, or egg, even when such substances as glycerine, sugars, amino-acids, fresh blood, etc., are added. It is absolutely essential that certain previously detailed bacteria or extracts from them be added to one or other of the media, before any growth of Jöhne's bacillus takes place, and this is equally true for strains of Jöhne's bacillus which have been freshly isolated from the animal body and for strains which have been cultivated on artificial media for 15 months or more.

Of the bacilli tested, undoubtedly the most suitable for adding to the medium is the timothy-grass bacillus; certain strains of human tubercle bacilli are also very good.

On the egg timothy-grass bacillus medium previously described, Jöhne's bacillus, when taken from the animal body, will grow as tiny discrete colonies which usually become visible in three to five weeks. At first the colonies are round, smooth, and dull stone white, they are slightly heaped up, and as growth increases this becomes more marked, while the colour turns to a dull light yellow. Later, the colonies may coalesce and the growth show some wrinkling, while the colour may change to a light yellowish brown. The degree of pigmentation seems to be considerably influenced by the amount of pigment in the timothy grass bacillus incorporated in the medium, and possibly also, though to a less extent, by the colour of the egg itself.

If the first culture taken from the diseased tissue is sub-cultured on to a fresh tube of medium, visible growth occurs on this in a few days, and may reach its maximum in about two months instead of three. The growth now appears as a heaped-up continuous line along the needle track, with only an occasional discrete colony at the margins. Wrinkling of the surface may also be more marked, especially if the culture is growing very well on a tube which is a little dry. When the timothy-grass bacillus in the medium is replaced by any of the other suitable bacilli or bacillary extracts, the growth of Jöhne's bacillus does not materially differ from the description given, except in rapidity and amount, and also in the degree of pigmentation. When inoculated into fluid media containing a suitable bacillus or bacillary extract, such as ordinary glycerine peptone bouillon, made alkaline, and containing ½ per cent. of alcoholic extract of the timothy-grass bacillus, Jöhne's bacillus grows as tiny whitish grains which settle to the bottom of the tube or flask. These gradually increase in size and number, ultimately reaching the dimensions of a millet seed. There is no general turbidity of the medium; and with bacilli freshly isolated from the animal body, no film formation. With one of our strains we have recently obtained some growth on the surface of the fluid media, and we expect, in course of time, to obtain a similar growth with the other strains.

Vaccines analogous to Koch's new tuberculin and Wright's vaccines can also be prepared, but so far we have not had an opportunity of testing the curative effect of these, and, even if successful, the cost of administering such vaccines would prevent their general use. So far, we have been unable to prepare a diagnostic vaccine of sufficient strength to give a positive result with pseudo-tuberculous enteritis, but we anticipate that it will be possible to prepare such a vaccine with the bacillus growing more vigorously on the surface of fluid media.

Cultures are not easily killed by diffuse daylight, as we have had them standing before a window on the bench for some weeks without any apparent harm.

Jöhne's bacillus would also appear to be fairly resistant to the action of disinfectants, since two of our strains were isolated from material which had been subjected to the action of a 1-per-cent. watery solution of ericolin at 37° C. for two hours. In this respect it is no less resistant than the tubercle and lepra bacilli.

## *Conclusions.*

From the experiments detailed in this paper it is possible to deduce certain conclusions, the most important of which are the following :—

1. The acid-fast bacillus present in cases of pseudo-tuberculous enteritis of bovines, and known as Jöhne's bacillus, fails to grow outside the animal body on any of the artificial media at present used by bacteriologists.

2. The bacillus shows no definite growth on fresh bovine tissue or fresh extracts of bovine tissue removed aseptically and placed into sterile tubes.

3. There is no evidence that Jöhne's bacillus grows in symbiosis with an ultra-microscopic virus.

4. The specific bacillus will grow on media containing the dried and powdered growth of certain acid-fast bacilli which have been previously killed, and this is so even when the dead bacilli have been kept for a period of eight years, and subjected to a temperature of 115° C. in the autoclave for 1 hour.

5. The most suitable bacillus to incorporate in the medium is the timothy-grass bacillus, and to a somewhat less degree the smegma bacillus of Moeller and the nasenschleim bacillus of Karlinski. The human type of tubercle bacillus is also good, but on media containing the avian type Jöhne's bacillus grows very slightly, if at all. With the few bovine strains tested in media we were unable to get any definite evidence of growth with Jöhne's bacillus. Tubercle bacilli isolated from cats also gave negative results.

6. The essential substance or substances necessary for the growth of Jöhne's bacillus can be extracted from the various acid-fast bacilli which give positive results by means of hot ethyl alcohol.

7. We have isolated Jöhne's bacillus from five consecutive cases of pseudo-tuberculous enteritis, and have proved the morphological and biological characters of the bacilli isolated to be identical in every respect.

8. The bacilli isolated produce no lesions in mice, rats, guinea-pigs, rabbits, pigeons, or hens, if given by the mouth or inoculated into the peritoneal cavity or into a vein or subcutaneously.

9. The specific bacillus, when inoculated intravenously or given by the mouth to bovines, reproduces pseudo-tuberculous enteritis in the animal, and this cannot be distinguished from the original disease either clinically during life or *post mortem*. Further, the bacillus can be recovered from the lesions in the intestine of the inoculated animal, and shows characters in every way identical with the bacilli isolated from the original cases.

10. Animals suffering from pseudo-tuberculous enteritis, either normally contracted or experimentally produced by the inoculation of pure cultures of Jöhne's bacillus, give no definite reaction with diagnostic vaccines prepared from cultures of the timothy-grass bacillus or from the avian tubercle bacillus.

11. Vaccines can be prepared from cultures of Jöhne's bacillus similar to those prepared from other acid-fast bacilli.

12. Diagnostic vaccines prepared from cultures of Jöhne's bacillus grown on tubercle bacillus medium gave positive reactions with tubercular animals, which proved the medium used to be unsuitable for the preparation of a specific diagnostic vaccine for pseudo-tuberculous enteritis.

13. Vaccines prepared from cultures of Jöhne's bacillus on a timothy-grass bacillus medium gave negative reactions with normal and with

tubercular animals, and also with bovines suffering from pseudo-tubercular enteritis. We believe this to be due, partly to the small amount of growth in the fluid media, and partly to the fact that most of the growth was obtained from solid media and therefore not made in the same manner as diagnostic tuberculin. We also believe that a highly concentrated vaccine will be required, and that we shall be able to prepare this now that one of our strains of Jöhne's bacillus has started to grow on the surface of fluid media containing the timothy-grass bacillus.

In conclusion we may say that we are greatly indebted to Mr. de Vine, Mr. Hamilton, and Dr. Bulloch for the specimens and materials they have given us, to the donor of a naturally infected bull, and especially to the Royal Society for the Government grants which enabled us to purchase the other animals. An investigation such as this requires a large number of bovine experiments, but these we have been unable to perform through lack of funds. Insufficient apparatus in the earlier stages also caused us considerable delay in the preparation of an efficient diagnostic vaccine, and we had no animals on which to test the first vaccine prepared. We now possess the necessary apparatus, and a number of vaccines are in course of preparation, but unless the animals on which to test the vaccines are forthcoming, we shall be unable to prove the efficacy or otherwise of the vaccines, and shall be forced to leave it to other workers more favourably placed financially. In any case it is to be hoped that other workers will test a diagnostic vaccine prepared in the manner we have indicated, for the economic loss from pseudo-tuberculous enteritis in such places as Denmark and the Channel Islands is of a very serious nature.

Some months ago we sent sub-cultures of the specific bacillus which we had grown to various workers in the British Isles, France, and in Denmark, and we hope that the desired vaccine will soon be obtained.

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# *On the Fossil Flora of the Forest of Dean Coalfield (Gloucestershire), and the Relationships of the Coalfields of the West of England and South Wales.*

By E. A. Newell Arber, M.A., F.L.S., F.G.S., Trinity College, Cambridge,
University Demonstrator in Palæobotany.

(Communicated by Prof. T. McKenny Hughes, F.R.S. Received November 18,
1911,—Read February 1, 1912.)

## (Abstract.)

Very little has been previously recorded of the flora of the Forest of Dean coalfield, and in the present paper the results of a thorough examination of the flora, and of the vertical distribution of the plants in the three divisions of the productive measures of this coalfield, are discussed. In all 44 species are described, none of which, however, are new to Britain, though some are rare plants elsewhere. The list of species, which have been collected chiefly by the aid of grants from the Royal Society Government Grant Committee, is as follows :—

EQUISETALES—

*Calamites varians* Sternb.; *C. ramosus* Artis.; *C. suckowi* Brongn.; *C. undulatus ?* Sternb.

*Calamocladus equisetiformis* (Schloth.).

*Annularia radiata ?* (Brongn.); *A. stellata* (Schloth.); *A. galioides* (L. and H.); *A. sphenophylloides* (Zenker).

*Calamostachys tuberculata* (Sternb.).

*Macrostachya infundibuliformis* (Brongn.).

SPHENOPHYLLALES—

*Sphenophyllum emarginatum* Brongn.; *S. majus* (Bronn).

PTERIDOSPERMEÆ AND FILICALES—

*Sphenopteris neuropteroides* (Boulay); *S. (Renaultia) chærophylloides* (Brongn.).

*Mariopteris muricata* (Schloth.); *M. latifolia ?* (Brongn.).

*Neuropteris scheuchzeri* Hoffm.; *N. macrophylla* Brongn.; *N. ovata* Hoffm.; *N. rarinervis* Bunb.; *N. (Cyclopteris) fimbriata* Lesq.

*Alethopteris aquilina* (Schloth.); *A. grandini* (Brongn.); *A. davreuxi* (Brongn.).

*Pecopteris miltoni* (Artis.); *P. polymorpha* Brongn.; *P. arborescens ?* (Schloth.); *P. (Dactylotheca) plumosa* (Artis.).

SEMINA INCERTÆ SEDIS—

*Trigonocarpus nœggerathi* (Sternb.).

LYCOPODIALES—

*Lepidodendron lanceolatum* Lesq.; *L. aculeatum* Sternb.; *L. wortheni* Lesq.; *L. dichotomum* Sternb.

*Lepidophloios cf. L. laricinus* Sternb.

*Lepidophyllum majus* Brongn.; *L. brevifolium* Lesq.

*Sigillaria lævigata* Brongn.; *S. elongata* Brongn.; *S. rugosa* Brongn.; *S. trigona* Sternb.; *S. tessellata* (Sternb.); *S. brardi* Brongn. var. *denudata* (Gœpp).

CORDAITALES—

*Cordaites angulosostriatus* Grand' Eury.

The floras of the three divisions of the productive measures in the Forest of Dean are compared, and it is found that they are practically identical. All three divisions belong to the palæobotanical horizon known as the Upper Coal Measures. It is shown that there is a marked agreement between the flora of the Forest of Dean and the Upper Coal Measure floras of other British coalfields, though the following species which occur in the Forest have not previously been recorded from this horizon elsewhere:—

*Annularia galioides* (L. and H.).

*Sphenophyllum majus* (Bronn).

*Mariopteris latifolia ?* (Brongn.).

*Lepidodendron dichotomum* Sternb.

*Sigillaria rugosa* Brongn.; *S. trigona* Sternb.; *S. brardi* Brongn. var. *denudata* (Gœpp).

The flora of the Forest of Dean is contrasted with those of the neighbouring coalfields. As compared with the Radstock flora, there is found to be a general agreement, though there are important differences in detail, which are more marked than those which exist between the known floras of Radstock and Bristol. These differences, however, do not appear to indicate any considerable disagreement as regards the horizon, for the percentage of Stephanian plants present is approximately the same in each case. They are best explained as local variations in the distribution of the flora of the period.

The horizon of the so-called Millstone Grits, below the Upper Coal Measures and above the Carboniferous Limestone, is discussed. Reasons are advanced in support of the view that the Upper Coal Measures of the Forest overlie unconformably the so-called Millstone Grits, which in reality are the higher beds of the Carboniferous Limestone series, which here have an arenaceous facies. True Millstone Grits, as well as Lower, Middle, and Transition Coal Measures, are absent in the Forest of Dean.

The relationships of this coalfield to the neighbouring coalfields of the West of England and South Wales are discussed from the palæobotanical standpoint. It is found that the Forest of Dean basin exhibits no obvious relationship, either to the South Wales or to the Radstock–Bristol coalfields.

The Pennant Grits of South Wales belong to a lower horizon than the markedly arenaceous series (the "Forest of Dean stone") of the third division of the Forest. The Radstock–Bristol and Forest of Dean basins are believed to be related tectonically, though not to the main axes of South Wales and the Mendips, but to a secondary cognate uplift, stretching north and south, and approximating to the valley of the Severn. On the other hand the Forest of Dean does not appear to be related to the Welsh borderland series of coalfields, stretching from Newent to Shrewsbury.

In the case of the Forest of Dean, it seems evident that the Lower Carboniferous rocks and the Old Red Sandstones of the area remained elevated above sea level, and were denuded until the beginning of Upper Coal Measure times, whereas in South Wales depression and deposition set in in Middle Coal Measure times, and in the Radstock–Bristol area during the Transition Coal Measure period. Thus, on the palæobotanical evidence, the relationships of the coalfields of the West of England and South Wales have proved to be more complex than has hitherto been supposed, and this appears to be due in part at least to the coincidence of three distinct axes of elevation in the neighbourhood of the Forest of Dean.

# *Simultaneous Colour Contrast.*

By F. W. Edridge-Green, M.D., F.R.C.S., Beit Memorial Research Fellow.

(Communicated by Prof. E. Starling, F.R.S. Received December 4, 1911,—
Read February 1, 1912.)

(From the Institute of Physiology, University College.)

The subject of colour contrast presents exceptional difficulties because of the number of factors to be taken into consideration. It is necessary to eliminate the effects of successive contrast. Many of the results which have been put down to simultaneous contrast are really due to successive contrast. The surfaces to be compared should either be viewed by a flash of light of very short duration or by one eye which is kept rigidly fixed upon a definite point.

In a series of important papers Hering* has shown that the explanation of contrast given by Helmholtz is not tenable. I hope to show that another explanation is possible which is even more in accordance with the facts. I propose to review some of the experiments of Hering and to show that in conditions in which, according to the requirements stated by him, colour should be visible no colour is to be seen.

In experiments on simultaneous contrast it is necessary in order to avoid effects of luminosity contrast to have the two surfaces as nearly as possible of the same luminosity. It is also necessary in dealing with mixed colours such as those formed by the light reflected from pigments to take into consideration the effects of chromatic aberration. When a surface reflects lights of different wave-lengths these lights are not all brought to the same focus on the retina. Diffusion circles will extend on both sides of the image of the coloured object on the retina and will influence the colour of another image immediately adjacent. I have made a mosaic of small pieces of coloured cardboard and the effect of the mixture of lights is very noticeable. In general each colour differs as it would do if the other colour had been objectively added to it. These colour changes have been mistaken for effects of simultaneous contrast.

## *Coloured Shadows.*

In the classical experiment which has been the subject of so much discussion an opaque object is placed upon a white surface and illuminated on one side by daylight and on the other by a candle or petroleum lamp.

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* 'Pflüger's Arch.,' 1886, 1887, 1888.

By moving the candle the relative luminosities of the two shadows can be so adjusted as to appear similar in brightness.

The shadow thrown by the candle and which is illuminated by daylight appears blue, whilst that formed by the daylight and illuminated by the reddish-yellow candle light appears yellow. It will be noticed that the shadow of the candle which is illuminated by white daylight is really grey, as it is only illuminated by a white light. Helmholtz and Hering therefore have referred to the blue coloration of the shadow as the subjective blue. I propose to show that the blue coloration of the shadow is relatively objective blue in the circumstances of the experiment.

It must be noted that the white surface on which the opaque object is standing and which is free from any shadow is illuminated by both daylight and candle light. Though it is still considered as a white surface it is really objectively yellow, to the extent of the added amount of candle light in the total amount of candle light and daylight which is reflected from the white surface, the degree of objective yellowness amounting to the difference between candle light and daylight in the proportion of the two. The blue shadow is therefore relatively blue in comparison to this white surface reflecting both lights when this surface is set up as a standard of white. In the same way the yellow shadow is relatively yellow in comparison to the whole surface. It must be noticed that daylight is not a fixed unalterable white but differs considerably according to the time of day and source; the light reflected from the sky is much bluer than that of direct sunlight.

All our estimations of colour are only relative and formed in association with memory and the definite objective light which falls upon the eye. In many of the most striking contrast experiments the colour which causes the false interpretation is not perceived at all: for instance if a sheet of pale green paper be taken for white a piece of grey paper upon it appears rose-coloured, but appears colourless when it is recognised that the paper is pale green and not white.

If, in repeating the experiment with coloured shadows, the opaque object be placed upon a dull black surface and two pieces of white paper be placed on this surface for the shadows, care being taken that these pieces of paper are the exact size of or smaller than the shadows, these will appear blue and yellow as before. If we now place a small dot on the paper on which there is a blue shadow and having covered one eye keep the other rigidly directed at this black spot whilst an opaque object is placed in front of the candle so that it no longer illuminates the paper or throws a shadow, both pieces of paper will appear white, being illuminated only by white daylight. The eye being still kept rigidly directed on the spot on the paper, the opaque object is removed so that the candle again throws a shadow on the paper. The shadow thrown by daylight immediately appears yellow and of greater saturation than before, but the shadow thrown by the candle appears white, as before, and without the faintest trace of a blue colour. The conditions are in every way favourable to the development of the blue colour, but none appears because the observer has been able to form a correct estimate of white. If the blue colour were a real subjective coloration caused by the yellow, blue should appear on the shadow from the candle.

The second experiment which is considered by Hering is that in which a small piece of grey paper is placed in the centre of a large square of coloured paper, and the whole is covered by a thin piece of tissue paper. The centre grey square becomes tinged of the complementary colour of the larger square on which it is placed. This experiment succeeds best when the colour of the ground is green. The grey paper is then tinged with the complementary colour, rose. This experiment, like most others of simultaneous contrast, has its effect much heightened if successive contrast be allowed to influence the result. In successive contrast the eye becomes fatigued for the colour particular to the rays of light which fall upon it.

I agree with Burch* that fatigue of the eye for any one colour does not increase its sensitiveness for any other colour. For instance, if the eye be fatigued for yellow the blue of the spectrum is considerably diminished, not increased. This is probably due to luminosity contrast. In the above experiment, therefore, it is very important that the eye should not see the grey square after having observed the green, as in that case it will be tinged with rose colour from successive contrast, because the eye has become fatigued for the green constituent of the white light reflected from it.

As is well known, the rose coloration is greatly diminished by using black or white instead of grey, or by isolating the grey square by drawing a black line round it. If, however, taking the greatest care that the eye be not moved, by steady fixation of the eye upon a black spot in the centre of the square for 10 seconds, and then looking at a sheet of white paper, a definite after-image is seen, a large rose-coloured square corresponding to the green paper, and a small green square in the centre corresponding to the small grey square, it will be noticed that the rose rapidly encroaches on the green, which disappears, whilst a rapid whirlpool appearance is seen in the centre of the field of vision. This experiment would seem to support strongly the view of Hering that the rose colour is actually subjectively produced on account of the proximity to green. I hope, however, to show by a simple experiment that this cannot be the explanation.

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* 'Phil. Trans.,' B, 1899, p. 5.

Let us consider the factors of the experiment when a grey patch upon a whitish-green ground is under observation: the objective light reflected from the grey patch differs from that on the green ground chiefly in containing less green, that is to say, the light is relatively and objectively rose-coloured in comparison with the whitish-green ground. The white light for this purpose may be divided into two portions, one of which is green and the other is a mixture of the remaining constituents of white light, which give rise to a sensation of rose. If a small portion of the common constituent green be deducted from both the whitish-green and the white the green will appear less saturated, and the white will appear rose. In this case the white light will be objectively rose in comparison to the green. It will be seen, therefore, that there are two ways in which the two coloured surfaces may be objectively considered. If the green be considered of less saturation than it really is, that is to say, a whiter colour, then the white will be rose in comparison with it, but if the white be considered white then the green will be objectively of greater saturation in comparison with it.

A simple experiment which I have devised for the purpose decides this point. If, when the grey square is situated upon the larger green square, another square of white paper of a size midway between the two other squares have a hole corresponding to the size of the small grey square cut in it, on laying this white paper so that the opening corresponds with the grey paper, the grey square will be seen without a trace of colour. A mark should then be made on the extreme left of the grey paper, and whilst one eye is kept rigidly fixed upon this spot the white square is gradually moved to the left until the field is occupied half by the whitish-green ground and half by the grey ground. It will be noticed that the green is greatly increased in saturation, and that not a trace of rose is visible upon the grey ground. If the right eye be kept fixedly upon these two surfaces for ten seconds, and then be directed to a white surface, a brilliant rose-coloured after-image, much brighter and more saturated than the one that was previously visible, and a pale white after-image, without a trace of colour corresponding to the grey region, will be seen. If the colour were really subjectively produced in the retina it should appear in this experiment as in the other. It might be thought that the increased saturation noticed in the green was due to the luminosity contrast of the white paper, but exactly the same result may be obtained with black paper, the green, when uncovered, appearing much more saturated, and the subsequent after-image formed on the white ground is rose colour for the green portion and dark grey for the white portion. In this case, as with white paper, not a trace of green is seen in the after-image of the grey.

A simple experiment described by Waller* illustrates this relativity of perception very well. If a strip of grey paper be placed upon a sheet of white paper, and then a piece of green paper be placed on either side of the middle third of the grey paper, and the whole covered with a piece of tissue paper, no contrast colour, or very little, will be visible. If, now, the middle third of the strip of grey paper be isolated by means of two pins placed transversely, the middle third becomes strongly tinged with the contrast colour, rose. On repeating this experiment, I find that, when the grey strip is seen as grey, the after-image is also grey, but when it is seen as rose, the after-image is green, and the rose after-image of the green appears less saturated. Also, when the contrast colour is developed, the objective green appears less saturated than when the grey strip appears grey.

A definite amount of saturation is necessary before a colour can be recognised. This colour becomes much more marked on contrast. A tinted paper which appears pure white without comparison may, when laid on a pure white surface, appear very definitely coloured. If a square of cream-coloured paper be placed on a white ground, it will appear of a decided pale yellow ; the colour of the white ground will, however, not be altered. If the after-image of this paper on a white surface be examined, it will appear as a pale blue square on a white ground, but no adjacent yellow is to be seen. The estimation of colour is always relative ; for instance, if a pale yellow diamond be given to a man, who has to classify diamonds, as a standard white, he will classify the pure white diamonds as blue, and not sufficiently estimate the amount of yellow in those diamonds which are yellow.

Our power of discrimination of colours is much more limited than is usually supposed. I have shown† that most persons can only differentiate about 18 separate regions in a pure spectrum, and that if one of these regions be examined with a double-image prism, so that the red side of one image be adjacent to the violet side of the other, no difference will be detected. Here we should expect that, if any colour induction were produced, an immediate difference would be observable between lights which are objectively so different.

In examining the two images, the greatest care should be taken to have them both of the same apparent luminosity. It will be noticed that, when the images are of different luminosity, the hue is also different. Both images appear monochromatic in themselves, but different in hue and luminosity. This is particularly noticeable in the blue-green region of the spectrum, as this is one of the portions of the spectrum in which the monochromatic divisions possess the fewest wave-lengths. The images are, however, larger, because of the increased separation of the wave-lengths in this region. When viewed with the double-image prism, a monochromatic division shows two monochromatic images side by side, and exactly similar in every respect when the intensity of both is similar, but, if the intensity of one be greater than that of the other, one will appear definitely blue and the other definitely green. I am inclined to think that Prof. Watson's results* are due to this cause, especially in association with stray light of different wave-lengths.

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* 'Journ. Phys.,' 1891, p. 44.

† 'Roy. Soc. Proc.,' B, 1911, p. 116.

This method is one which enables us to study very accurately the effects of simultaneous contrast. A monochromatic region can be viewed with the double-image prism, so that it appears as two images with a small space between. One of the shutters of the spectrometer can then be moved, so that the two images increase in size and just touch. It will be noticed that the effect of contrast is most apparent at the edges—for instance, if a yellow region be observed, one edge appears green and the other adjacent edge appears orange. The whole of the image appears to be altered, that is to say, the image at which the orange edge is seen appears to be more yellow throughout, and the green one more green throughout. Each appears as if it were moved further from the other in the spectral range. We can, however, make the same wave-length appear as different colours in the following way:—If a monochromatic region be isolated, for instance, yellow, no difference being detected, the whole of the wave-lengths occupying this region appear yellow. If, however, we take the wave-length occupying the central position of the region and move the shutter on the green side until it occupies this central position, we can then move the shutter on the red side until a fresh monochromatic region is observed. This appears absolutely uniform in colour, but the colour appears orange-yellow instead of yellow, including that portion that was previously seen as yellow. We can now move the shutter on the red side till it occupies the centre of the first-mentioned yellow region, and then, extending the shutter on the green side, form a fresh monochromatic region. The colour will appear absolutely uniform as before, but it has now changed, and the whole has become greenish-yellow.

The contrast colour is most developed when the surface on which it is seen is small and situated on a large surface of very pale colour which it is difficult to recognise as coloured without special comparison with a known white surface, as, for instance, if a small piece of grey paper be placed upon a large piece of very pale green paper. If the paper be regarded as white, the light reflected from the grey paper must be regarded as rose, for the subtraction of the small quantity of the green light from the light of the pale green paper in order to make this white is sufficient, when subtracted from the white light reflected from the grey paper, to make this appear rose. When it is recognised that the green paper is not white the contrast colour disappears, and the grey paper is seen as grey. The contrast colour is most developed on grey paper, and not nearly so well, if at all, on white or black paper. It is therefore produced in exactly those conditions in which the subtraction of a small quantity of green light will be most effective in altering the appearance of the colour. It is well known that in most contrast experiments, if a direct comparison be made with a known white surface, the contrast colour disappears. There is no reason why this should occur if the contrast colour were an actually induced colour.

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* 'Roy. Soc. Proc.,' B, 1911, p. 118.

My conclusion, therefore, is that the contrast colour developed in simultaneous contrast is due to the perception of an actual objective relative difference—in fact, the greatest difference which is perceptible in the circumstances, white being not a fixed objective quality, but a sensation produced by admixture of light of certain wave-lengths. If the sensation of one colour induce that of the complementary in the adjacent portion of the retina, there are many circumstances in which the colour ought to be visible and yet is not found. I have never yet, for instance (excluding negative after-images), seen the faintest trace of green on the dark surfaces in a photographic dark room illuminated by a pure red light. Neither have I come across any other person who has seen green in these circumstances. On coming out of the dark room white objects only appear slightly tinged with green, if any change be noticed at all. Not the faintest trace of green is to be seen round red lights at night, and I find the greatest difficulty in obtaining an after-image even by staring fixedly at the red light, if this be not of considerable intensity.

The subject of induction of colour by simultaneous contrast can be investigated in another way, that is by entirely eliminating red or any other spectral colour and then studying the effects of simultaneous contrast. This may be accomplished by viewing objects through coloured glasses which are opaque to light of certain wave-lengths. In the case of red light we can use blue-green glasses, which are impermeable to the red rays. I have therefore had a pair of spectacles glazed with blue-green glass. This blue-green glass is absolutely opaque to the red rays from the termination of the spectrum to λ 646. There is considerable absorption from λ 646 to λ 588. The yellow rays are partially obstructed, whilst the glass is practically transparent to the green, blue, and violet rays. When objects are viewed through these spectacles not a trace of red is to be seen either directly or by contrast. An ordinary coal fire appears to consist of only yellow or yellow-green flames, no orange or red being visible. The yellow light of the spectrum or a yellow object adjacent to a green one still appears yellow, and the colour appears if anything to incline towards green rather than red. Reds appear black, or in a very bright light, and when they reflect orange rays, a dull brown. Yellow, green, blue, and violet can be seen through the blue-green glasses. White objects at first appear blue-green, but after a short time again appear white. Objects corresponding to the dominant wave-length of the blue-green glass appear white or pale blue or pale green according to the composition of the colour. All contrasts are modified in a similar manner. For instance a grey square on a green ground, in circumstances which give a bright rose contrast colour, appears pale blue through the blue-green glasses, and a grey on a blue ground yellow-green. No colour is seen the light of which cannot pass through the blue-green glass. Sir W. Ramsay, whose vision on my classification* is trichromic (that is he describes the bright spectrum as consisting of red, red-green, green, green-violet, and violet), examined my series of contrasts through the blue-green glasses and in no instance called yellow red. He rather tended to call yellow yellow-green, or, to use the term which he prefers, green with a very small amount of red in it. It would appear, therefore, that the exaggerated simultaneous contrast which I have found to be characteristic of these cases is not found in the absence of the objective exciting light.

These facts point to the conclusion that the sensation of red is not produced by simultaneous contrast in the absence of objective red light. They also support the conclusion at which I had previously arrived from the study of colour fatigue† that yellow is a simple sensation and not compounded of a red and a green sensation. I can also find no evidence of the induction of colour by simultaneous contrast in the absence of objective light of that colour.

## *Summary.*

1. The colours seen by simultaneous contrast are due to the exaggerated perception of a real, objective, relative difference which exists in the light reflected from the two adjacent surfaces.

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* 'Hunterian Lectures on Colour Vision and Colour Blindness,' Kegan Paul and Co. 1911, p. 24.

† 'Trans. Ophth. Soc.,' 1909, p. 211.

2. A certain difference of wave-length is necessary before simultaneous contrast produces any effect. This varies with different colours.

3. A change of intensity of the light of one colour may make evident a difference which is not perceptible when both colours are of the same luminosity.

4. Simultaneous contrast may cause the appearance of a colour which is not perceptible without comparison.

5. Both colours may be affected by simultaneous contrast, each colour appearing as if moved further from the other in the spectral range.

6. Only one colour may be affected by simultaneous contrast as when a colour of low saturation is compared with white.

7. When a false estimation of the saturation or hue of a colour has been made the contrast colour is considered in relation to this false estimation. That is to say the missing (or added) colour is deducted from (or added to) both.

8. A complementary contrast colour does not appear in the absence of objective light of that colour.

9. The negative after-images of contrasted colours are complementary to the colours seen.

# *An Alleged Specific Instance of the Transmission of Acquired Characters.—Investigation and Criticism.**

By T. Graham Brown (Carnegie Fellow).

(Communicated by Prof. C. S. Sherrington, F.R.S. Received December 9, 1911,—Read February 15, 1912.)

(From the Physiological Laboratory of the University of Liverpool.)

CONTENTS.

PAGE
I.—Historical Note ... 555
II.—Experiments Concerning the Nature and Cause of the Brown-Séquard Phenomenon—
  *a.* The Views held by previous Observers ... 559
  *b.* The Occurrence of the Phenomenon in Relation to Trophic Changes in the Feet ... 560
  *c.* The Occurrence of the Phenomenon in Relation to Changes in the Great Sciatic Nerve ... 561
  *d.* Occurrence of the Phenomenon in a Case of Accidental Injury to the Foot ... 562
  *e.* Phenomena under Narcosis in the Guinea-pig and other Animals ... 563
  *f.* Phenomena in Decerebrate and in Decapitate Animals ... 564
III.—Conclusions Concerning the Nature of the Brown-Séquard Phenomenon ... 566
IV.—Conclusions Concerning the Cause of the Brown-Séquard Phenomenon ... 567
V.—Experiments on Transmission of the Phenomenon—
  *a.* Experiments on Guinea-pigs ... 571
  *b.* Experiments on Rats ... 571
VI.—Conclusions Concerning the Alleged Transmission of the Brown-Séquard Phenomenon ... 572
VII.—Summary ... 573
Table of References ... 577

## I. *Historical Note.*

The adherents of the theory of the transmission of acquired characteristics rely upon a comparatively small amount of experimental evidence. Of that evidence, "Brown-Séquard's epilepsy" in guinea-pigs is considered an important part.

Brown-Séquard discovered that guinea-pigs develop "epilepsy" after certain lesions of the nervous system, and he stated that the young of such "epileptic" animals may also exhibit the "epilepsy," although this state does not occur in normal individuals.

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* The expenses of this research have been defrayed by a grant from the Carnegie Trust. The experiments in part were performed in the Physiology Laboratory of the University of Glasgow.

His first papers date from the year 1850 (1, 2, 3, 4, 5), and he continued to investigate the phenomenon for more than 40 years, publishing his results in a series of papers and short notes.*

He found that the condition might be produced by means of many different lesions. Of these, it is necessary here to refer only to section of the great sciatic nerve (9, 11, 12, 13); to amputation of the leg, that is, section of the various nerves of the leg (18); and to section of the lumbar posterior spinal roots, which supply the great sciatic nerve (12).

A detailed account of these experiments, in so far as they concern the phenomenon, and not its transmission, has been given by the author in another place (45).

The condition may be described as it occurs after removal of a part of one great sciatic nerve. At a variable time after the production of the lesion, a rapid scratching movement of the hind limb of that side is evoked on gently pressing the skin of the face upon the same side as the lesion. The pressure must be applied within a definite area—the "epileptogenous zone" of Brown-Séquard—and is ineffective if applied to the corresponding area of skin upon the other side of the body. This reaction constitutes the "incomplete attack." The scratching is accompanied by a twisting of the neck and back.

As time elapses, after the appearance of this condition, the phenomenon enters upon a second phase—that of the "complete attack." Here the twisting of the back and neck becomes more pronounced, and the animal loses its balance. At a point in the phenomenon the sense of the bending in back and neck reverses, and the loss of balance is possibly a result of this. With the change in the direction of the curvatures, the scratching movements usually cease in the hind limb of the side of the lesion, and then appear in the opposite hind limb. The scratching shortly again changes sides, and so the movements alternate from side to side of the body. This "attack" may continue for a minute or more after the mechanical stimulation of the skin of the face or neck has ceased (fig. 1).

Brown-Séquard has been confirmed by many observers in his general description of the appearances of this condition. Two points may be especially noted. The first of these is that the phenomenon is a clearly recognisable entity, and the second is Brown-Séquard's statement that, although he had had many thousands of guinea-pigs under observation, he had never observed the condition save in those especially operated upon (11, 25).

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* In the absence of a satisfactory bibliography the search for Brown-Séquard's original communications necessitates much labour. I have therefore given a list of his most important publications at the end of this paper. References to these papers in the text are indicated by numbers in brackets. This list supplements another given by the author (45), in which the papers deal with the physiological aspect of the condition. Neither list is complete, for I have probably not read all the original papers, and many of those which I have read it was unnecessary to quote.

Apart from the phenomenon itself, two other consequences of a section of the great sciatic nerve are of interest. In the first place, the special area of skin usually becomes infested with lice (6, 7, 12); and in the second place the animals often acquire the habit of nibbling the anæsthetic parts of the foot.

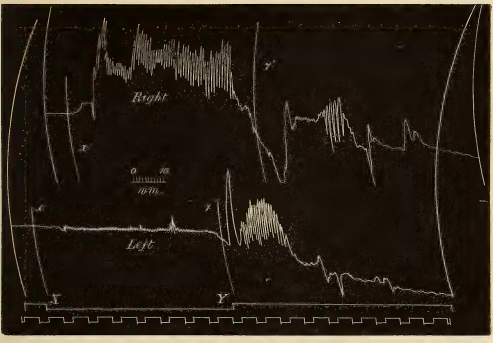

FIG. 1.—February 14, 1910. Record of a "complete" Brown-Séquard reaction in a guinea-pig. The mechanical stimulus was applied to the right side of the neck between the points X and Y of the signal line (ordinates $x$, $x'$ : $y$, $y'$). Between these points the scratch was confined to the right hind limb, and had this been the only reaction the phenomenon would have been "incomplete." But it will be observed that, on cessation of stimulation at Y, scratching movements appear in the left hind limb, then cease and reappear in the right hind limb—thus producing a "complete" reaction. The record indicates flexion as a rise and extension as a fall of the curve. The rapid up-and-down movements indicate the "beats" of the scratch.

In 1860 (8) Brown-Séquard stated that this peculiar phenomenon might appear in the offspring of affected guinea-pigs. The paper is simply a note, in which he declares that he had seen the transmission in six cases, and that the primary injury was to the spinal cord. This statement was accepted by

Darwin in 1868 (10), and in 1870 Brown-Séquard published a number of short notes on the subject. The condition might be transmitted from the father only, and the mother might acquire the condition from the father (14). In seven instances he observed malformation of the feet in the offspring of parents, male or female, which had the phenomenon. In several of the offspring there was an "epileptogenous zone" and "incomplete epilepsy" (15, 16, 17). Another animal born of an "epileptic" mother had loss of toes and became "epileptic"—probably because of an alteration in a sciatic nerve (19).

In 1871 Westphal, who produced the state of "epilepsy" in a different manner, confirmed the transmission of the condition to the offspring (23, 24), the attacks being "incomplete."

Brown-Séquard later gave greater details of the phenomenon of transmission. Thus, in 1875, he gives a list of the various conditions which he has observed to be transmitted (25). In this, he includes transmission of the state of "epilepsy" from "epileptic" parents whose sciatic nerves had been cut, and transmission of malformation of the feet from similar parents. He states that he has never seen "epilepsy" in normal guinea-pigs, although he has had many thousands under observation, and he also says that the transmission of malformation of the toes is very rare—he has only recorded 13 cases—and that the inheritance of "epilepsy" is only seen in such cases. Another paper, in 1882, adds little new (32). He, however, states that he had, at the time, 20 young guinea-pigs, the offspring of parents in which the sciatic nerve was cut, and that these offspring had muscular atrophy of the legs in consequence.

Other observers have investigated the problem of this transmission. Brown-Séquard states that his pupil Dupuy had confirmed him (32). Westphal, as we have seen above, did also (23, 24).

Romanes (35) says that "epilepsy" produced by lesions of the cord and of the sciatic nerve is seldom inherited, and he also states that the malformation of the feet produced in operated animals by their habit of nibbling the parts of the feet rendered anæsthetic by section of the great sciatic nerve did not appear in the offspring he observed through six generations.

Obersteiner (26) confirmed the transmission of "epilepsy" in guinea-pigs, but more recently Max Sommer (38) failed to do so; and Bramwell and Graham Brown (42) also obtained purely negative results in the case of 10 young, the offspring of "epileptic" parents, which they examined.

Lately, Taft (52) has failed to observe "epilepsy," or abnormality, in 114 offspring of "epileptic" parents, neither did these appear in the third and fourth generations. He lays stress upon the observation of Brown-Séquard that the young which exhibited "epilepsy" had, in all cases, also malformation of the toes.

Maciesza and Wrzosek (56, 57, 58) have, still more recently, taken up the investigation afresh. They found that of 82 young guinea-pigs born of "epileptic" parents, it was possible in 33 cases to induce "incomplete epileptic" attacks by mechanical pressure applied to the skin of the neck and face. They never obtained "complete" attacks. They also found that in the case of young guinea-pigs, born of normal parents, it is sometimes possible to induce similar "attacks." But, at the same time, they observed that the date of the first appearance of either "incomplete" or "complete epilepsy," after section of one great sciatic nerve, is one week earlier, in the case of the offspring of "epileptic" parents, than it is in the case of normal guinea-pigs.

The same observers also investigated the possibility of the inheritance of feet malformations, produced by section of the great sciatic nerve, in guinea-pigs and in white mice. In the large number of offspring observed—in the case of the mice over several generations—they never saw malformations of the hind limbs. But, in the offspring of normal parents, they observed a small number of cases where there were such malformations, in most cases acquired. Amongst the offspring of normal guinea-pigs, the percentage of cases in which there were malformations of the lower limbs—either acquired or inborn—was between one and two; this percentage corresponds very closely to that found by Brown-Séquard in the offspring of "epileptic" parents.

In looking back over these results we must admit that certain observers have undoubtedly observed the presence of the "complete" Brown-Séquard phenomenon in the offspring of parents in which the condition was also present and caused by injury to the great sciatic nerve; and that at the same time, and in the same offspring, there were malformations of the lower extremities. We must also admit, however, that such malformations of the hind limbs may be present, too, in the offspring of normal parents.

## II. *Experiments Concerning the Nature and Cause of the Brown-Séquard Phenomenon.*

(a) *The Views Held by Previous Observers.*—Brown-Séquard classified the phenomena (22) as either "double" or "single," and as either "complete" or "incomplete." When the phenomenon is "single," the scratching movements are confined to the hind limb of that side on which the great sciatic nerve was cut. In the case of the "double" phenomenon the scratching movements first occur upon that side, but later spread to the other side. The terms "complete" and "incomplete" refer respectively to the loss of consciousness or the absence of loss of consciousness which Brown-Séquard described. Thus a "double" attack may yet be "incomplete" according to him. But the phenomenon of loss of consciousness cannot accurately be investigated, and it is better to use the terms "complete" and "incomplete" in the sense in which Brown-Séquard used "double" and "single." This has been the practice of the present author in previous papers, and in it he has been followed by Taft.

Brown-Séquard seems to have considered these different varieties of the phenomenon as essentially of the same nature, and he states (36) that "epilepsy" which exists in guinea-pigs after the various lesions is absolutely equivalent to ideopathic epilepsy or epilepsy of cerebral origin in man. He thought (21, 22) that the primary cause of the former condition was the irritation of the central stump of the cut sciatic nerve. The secondary alterations (of the posterior columns of the spinal cord on the same side, of the hair in the "epileptogenous zone" on the face and neck, and presumably the "epileptic faculty" itself) do not depend upon the transmission of the morbid organic state by continuity of tissue, but upon the propagation of a morbid influence exercised at a distance by the irritated fibres of the central stump of the cut nerve.

Weismann (34, 41) apparently accepted Brown-Séquard's view of the nature of the condition, for he states that epilepsy is not a morphological character but a disease, and he supposes that the condition might be caused by an unknown microbe which might pass to the young, and in them produce the same phenomena.

This view was vigorously combated by Brown-Séquard (37), who failed to find a specific micro-organism. He also points out that the condition never follows certain lesions (such as section of the brachial plexus), although it always follows certain other lesions of a similar nature (section of the trunk of the great sciatic nerve). And, in addition, the phenomenon may appear when the sciatic nerve is injured without breaking the skin, as when it is involved in the callus thrown out when there is a fracture of the bones.

(b) *The Occurrence of the Phenomenon in Relation to Trophic Changes in the Feet.*—After section of the great sciatic nerve, a large part of the surface of the skin of the foot and leg is rendered anæsthetic. Guinea-pigs in which this anæsthesia is produced commonly acquire the habit of nibbling the insensitive parts until they may destroy the two outer toes, or even a greater part of the limb. This habit may be controlled by placing fluids which have a bitter taste upon the foot. It is hardly necessary to say that the animal itself can feel no pain.

As it might be argued that bacterial infection from the raw surface may be the primary cause of the appearance of the condition, it is interesting to enquire whether the phenomenon may appear in the absence of this "trophic" change.

In illustration of this the three following experiments may be quoted:—

*Guinea-pigs* 60, 61, 63.—These three animals had a portion of their right great sciatic nerves removed on June 11, 1909. In each case the "incomplete" reaction of the Brown-Séquard phenomenon appeared on or before July 10, 1909. Two days later records of the movements of the hind limbs were recorded. At that time there were no traces of "trophic" change in the right foot of 60. In the case of 61 there was only a slight change in one claw, and in the case of 63 there was a well-marked "trophic" change of the right foot. There was no very great difference between the records taken from the three animals. The best records were obtained from 60 and 63.

These experiments seem to prove that the presence or absence of a "trophic" change in the foot is not a determining factor in the production of the phenomenon.

In connection with the "trophic" changes in the foot, another observation may be recorded. The change is in great part due to the habit of nibbling the anæsthetic parts. This may be regarded as a morbid habit. Not only does a guinea-pig nibble its own foot, but it may nibble the foot of another. Protective reflexes prevent this from being effective when that other animal is normal. But if it, too, have its foot rendered anæsthetic by section of the great sciatic nerve, then the nibbling may be effective in destroying part of the foot. Thus it has been noticed that if two such individuals be kept in the same cage, it sometimes happens that they will not only nibble their own feet, but will also nibble each other's feet. This acquired habit, then, may lead a guinea-pig to the unnatural act of devouring others of its own kind, and may explain in part the greater than normal destruction of young by the mother in the case of such individuals.

(c) *Occurrence of the Phenomenon in Relation to Changes in the Great Sciatic Nerve.*—When the great sciatic nerve is severed and a portion removed, regeneration does not occur, and the central stump forms a bulbous enlargement. It might reasonably be argued that a continued irritation arising in this stump might be the cause of the production of the phenomenon. To investigate this point the nerve, in a guinea-pig which demonstrated the phenomenon, has again been cut central to the first lesion.

*Guinea-pig* 21.—In this experiment the great sciatic nerve was divided and a portion removed on January 8, 1909. This lesion was made at the level of the middle of the thigh. In the beginning of July of the same year tracings of the scratching movements—both of the Brown-Séquard phenomenon and of the narcosis scratch—were recorded, and the great sciatic nerve was again divided and a part removed at the level of the great trochanter of the femur. In this second operation the narcosis scratch occurred, and was recorded graphically both before and after the division of the nerve. Subsequent to the operation the scratching movements of the Brown-Séquard phenomenon might still be obtained and were recorded.

At the time of the second operation the Brown-Séquard phenomenon was "incomplete." The rhythm of the "beats" of the scratching movements was between 11 and 13 per second. After the second operation it was slightly more difficult to induce the phenomenon, and the rhythm of the beats was the same.

The scratching movements of the narcosis scratch before the second operation occurred chiefly upon the side which exhibited the Brown-Séquard phenomenon (the right side of the animal). After the operation the same was the case. There was no important difference between the records taken before and after the second division of the nerve, and the rhythms of the "beats" were similar.

This experiment shews that a continued irritation arising from the central stump of the severed nerve is not the cause of the Brown-Séquard phenomenon.

(d) *Occurrence of the Phenomenon in a Case of Accidental Injury to the Foot.*—Brown-Séquard noticed that accidental injury of the nature of fracture of the bones might produce the phenomenon. He attributed this effect to the implication of the nerve in the callus produced during the process of repair of the broken bones.

The following instance of the appearance of the phenomenon after another kind of accidental injury is of some interest:—

*Guinea-pig* 55.—This animal was operated upon on May 7, 1909, for another purpose (section of the corpus callosum) not connected with the Brown-Séquard phenomenon. One morning, a few days later, it was found that a wisp of straw had become entangled tightly round one foot. This was at once removed, but it was found that the foot had become anæsthetic, and the foot subsequently became swollen. As the animal obviously suffered no inconvenience and was perfectly healthy it was decided to keep it. The foot was carefully treated, but sensation was not restored and it withered. On July 2, 1909, a well-marked "complete" Brown-Séquard phenomenon could be obtained on either side by stimulation of the skin of the face or neck.

(e) *Phenomena under Narcosis in the Guinea-pig and other Animals.*—In 1909 the present author described certain phenomena which occur under

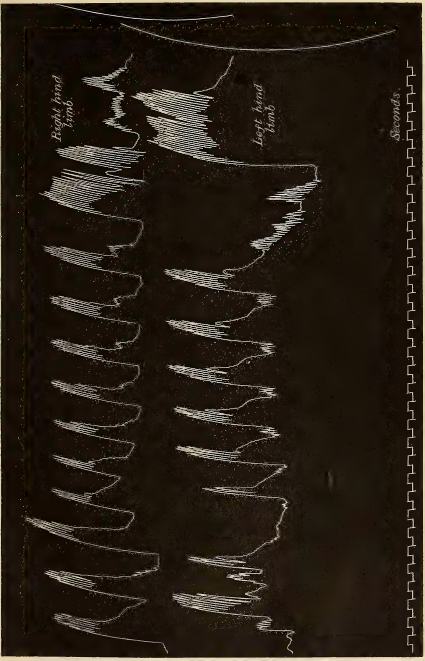

Fig. 2.—February 7, 1910.—Record of the rhythmic alternating scratching phases of the "narcosis scratch" recorded from the right (upper tracing) and left (lower tracing) hind limbs of a guinea-pig whilst in ether narcosis.

narcosis in the guinea-pig (46), and he has given more detailed observations in subseqent papers (47, 48, 51, 54, 55). These seem to have a direct bearing upon the question of the nature of the Brown-Séquard phenomenon.

Guinea-pigs and rabbits when under the influence of a general anæsthetic such as chloroform or ether exhibit peculiar scratching movements which alternate from one hind limb to the other (fig. 2).

The details of this phenomenon—which may be termed the "narcosis scratch"—have been given at length in previous papers and need not here be again repeated. But it may be said that suddenly a movement of scratching appears in one hind limb, and that at the same time the back is bent with its convexity to the opposite side, and the neck with its convexity to the same side. The movements of the scratch last thus for a few seconds, and then cease in that hind limb but appear in the opposite one. At the same time the bendings of the back and neck reverse, so that the animal assumes the mirror posture of that first described. In a similar manner the scratching movements in this limb cease and reappear in the first hind limb. And so the phenomenon proceeds, the alternation of scratching movements from side to side of the body lasting for several minutes in some cases. If the depth of anæsthesia be increased the movements decrease gradually in extent and finally die away. But if the depth of anæsthesia be decreased the movements cease suddenly.

Records of the movements obtained by the graphic method demonstrate their likeness to the movements of a true scratch-reflex. A relative difference lies in the rhythm of the component beats of the movement. In the "narcosis scratch" the rhythm is slower than in the scratch-reflex. An essential difference between them lies in the automatism and alternate repetition of the scratching phases of the "narcosis scratch." In them there is no ascertainable peripheral stimulus, and they continue automatically passing from one hind limb to the other.

(f) *Phenomena in Decerebrate and in Decapitate Animals.*—In the decapitate cat, as Sherrington has noticed (49), the scratch-reflex may easily be evoked by stimulation of the skin of the neck.

In the same preparation, however, "spontaneous" scratching may sometimes occur. During the making of this preparation a ligature is passed tightly round the neck of the carcase in order to prevent bleeding from the veins. It is probable that the "spontaneous" scratching is due to a mechanical stimulus set up by this ligature.

It occasionally happens that similar "spontaneous" scratching movements may continue for a considerable time and not upon the one side of the body only. In these cases the scratch alternates from one hind limb to the other, and the phenomenon—although its causation may probably be traced to a peripheral stimulus—resembles very closely the "narcosis scratch" in the guinea-pig.

Further, the two conditions are similar in the condition of the animals which exhibit them. Thus, in the decapitate cat, the higher centres are removed down to the level of the caudal margin of the medulla oblongata by operative procedure. In the guinea-pig under anæsthesia, certain of the higher centres are temporarily eliminated by the action of the narcotic.

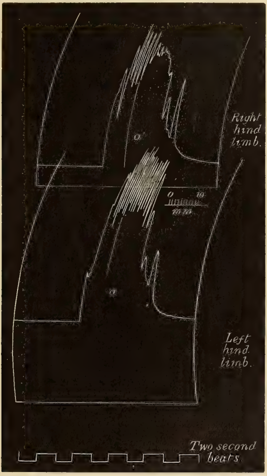

Fig. 3.—Record of the movements of the true scratch-reflex of the hind limbs obtained on stimulating the skin of the neck mechanically in a decerebrate guinea-pig. The ordinates *a*, *a'* mark corresponding points in the two tracings.

In the cat, as Sherrington has demonstrated (49), a state of asphyxia favours the scratch-reflex; and in the guinea-pig, as the present author has shewn (47), asphyxia favours the "narcosis scratch."

The present author at one time thought that a true scratch-reflex could not be obtained in the normal decerebrate guinea-pig (53). But he subsequently found (59) that it might appear in such preparations, although rarely. The observation is of interest. In a normal guinea-pig, from which the whole of the cerebral hemispheres had been removed, mechanical pressure applied to the skin of the neck evoked a distinct scratch-reflex in the hind limbs (fig. 3).

This observation demonstrates that a true scratch-reflex may be evoked in the guinea-pig. And a comparison between graphic records of it, and of the scratching movements of the Brown-Séquard phenomenon, demonstrates their similarity.

"Voluntary" scratching occurs in guinea-pigs as in most other mammals.

### III. *Conclusions Concerning the Nature of the Brown-Séquard Phenomenon.*

It is somewhat remarkable that comparatively little attention has previously been directed to the nature of the condition. This question is of great importance in relation to the value of the experiments in demonstrating the transmission of an acquired character. For it is obviously important to know if the phenomenon is one inherent in all guinea-pigs, and released by the special conditions of the experiments; or if it arises *de novo* as a result of the experimental interference.

The present author (45) has demonstrated the close similarity between the scratching movements of the Brown-Séquard phenomenon and the movements of the scratch-reflex as observed by Sherrington in the spinal dog (43, 44). Graphic records of the phenomenon in guinea-pigs demonstrate all the especial characteristics of the scratch-reflex.

We have now before us evidence of the characteristics of three scratching phenomena in the guinea-pig—the true scratch-reflex, the scratching movements of the Brown-Séquard phenomenon, and the scratching movements of the "narcosis scratch." The records, in general, exhibit the same characteristics in all three cases.

But more than this. In all three conditions there are concomitant phenomena, in other parts of the body, which accompany the scratching movements. Such are the bending of the back and neck, movements of the ears and eyes, movements of the fore limbs, etc. In all three conditions these also are present, although in differing degree.

It must, I think, be definitely admitted that the movements in the "incomplete" form of the Brown-Séquard phenomenon, in a scratching phase of the "narcosis scratch," and in the true scratch-reflex of the guinea-pig, are all exhibitions of the activity of one and the same mechanism. The "incomplete" Brown-Séquard phenomenon is, in fact, no more and no less than an example of the scratch-reflex in the guinea-pig.

The phenomena of the "complete" form of the condition at first sight raise certain difficulties. In the scratch-reflex of such a preparation as the spinal dog the scratching movements are usually confined to the hind limb of the side stimulated. Gergens (29) and, later, Magnus (50) have, however, shewn that the scratch may appear in the contralateral hind limb if the ipsilateral limb is restrained. The present author has observed the same phenomenon in a guinea-pig which only exhibited the "incomplete" Brown-Séquard phenomenon.

But in the "narcosis scratch"—a phenomenon, moreover, which appears in normal guinea-pigs—the scratching movements also alternate from side to side of the body.

It is therefore possible, and indeed probable, that the scratch-reflex is in a state of great potential excitability—upon that side of the body on which the great sciatic nerve has been cut—in a guinea-pig which exhibits the "complete" Brown-Séquard phenomenon. The first resultant of the effective stimulus will be to evoke the scratch-reflex in the hind limb of that side. At a certain point, however, the condition passes over into a state which parallels that condition seen in the "narcosis scratch."

From a consideration of these phenomena I think that there can be no reasonable doubt that the phenomena described by Brown-Séquard are special instances of states—the true scratch-reflex and the "narcosis scratch"—of which the conditioning mechanisms are inherent in all normal guinea-pigs.

If this be true, the Brown-Séquard phenomenon must not be regarded as a specific condition specifically created by the consequences of certain lesions of the nervous system, and thus as arising *de novo* in the animals so treated. It must rather be looked upon as being in itself an expression of the activity of a mechanism present in all guinea-pigs and only especially elicitable in consequence of the derangements produced by these lesions.

If the mechanism is present in all guinea-pigs it is equally present in the young of parents in which the Brown-Séquard phenomenon is present. But if this phenomenon is present in these young what is inherited as an acquirement is not the mechanism but the especial excitability of it.

### IV. *Conclusions Concerning the Cause of the Brown-Séquard Phenomenon.*

The question now arises—in what manner does the section of the great sciatic nerve produce the raised excitability of this mechanism?

Abel and Graham Brown have partially discussed this question (51). The special question examined by them was the causation of macroscopic and microscopic changes in the "epileptogenous zone" of skin from which the movements of the phenomenon are elicited by mechanical stimulation. This question concerns us here.

In this area of the skin the hair sometimes, but not always, becomes rough and broken and is shed. At the same time lice accumulate in the area and the skin is thickened.

In the case of one guinea-pig a very instructive observation was made. This animal exhibited a bare crescentic patch of skin behind the scapula of that side upon which the Brown-Séquard phenomenon might be elicited in consequence of the removal of part of the great sciatic nerve. This was certainly caused by excessive "voluntary" scratching directed to this very limited area. From this it was argued that the normal scratching movements were especially frequent upon the side of the lesion in guinea-pigs exhibiting the phenomenon. This supposition also rests upon direct observation. From this and other considerations it was concluded that the roughening of the hair (and the other changes in the special area of skin) are not caused directly by the section of the great sciatic nerve—either by an effect in producing a greater amount of scratching than is usual, or by an effect in producing a less amount of effective scratching in consequence of loss of the toes by the trophic changes in the foot—but that it might either follow indirectly a central change in the nervous system, or, what is more probable, be due indirectly to a lessened amount of effective scratching. This last supposition may be explained by supposing that the effect of the section of the nerve is to cause an increase in the excitability of the scratch-reflex evoked in response to the mechanical stimulation of a circumscribed part of the special area. When a normal stimulus falls outside this the scratch is at first directed to the stimulated point—is co-ordinate—but almost immediately irradiates to the point which is especially excitable. Thus all other areas receive inadequate grooming although the scratch-reflex itself may be described as relatively excitable.

The experiments described in a previous section (III, (b)) demonstrate not only that the "trophic" change in the foot is not the cause of the raised excitability seen in the condition, but that the possibility of effective or ineffective scratching (as regards only the state of the scratching implement) is not a determining cause.

That the scratch-reflex is especially excitable upon the side of which the great sciatic nerve has been divided is shewn not only by the occurrence of the Brown-Séquard phenomenon itself but also by evidence derived from the phenomena of the "narcosis scratch" as it appears in such individuals (47).

In these cases the "narcosis scratch" does not run its ordinary symmetrical course—alternating from side to side of the body. More usually it occurs at first only upon the same side as that which exhibits the Brown-Séquard phenomenon. The movements may be confined entirely to this side, but in other cases later in the experiment they appear upon the opposite side.

The section of the great sciatic nerve causes two kinds of change in the individual. Of these the first is that peripheral to the place of section of the nerve. There is paralysis of certain of the muscles of the lower limb (extensors and flexors of the ankle and the peroneal muscles and the short muscles of the foot), and anæsthesia of a certain peripheral field which indirectly produces a loss of the toes. The second change is central. The excitability of the scratch-reflex is raised, and at the same time there is a parallel change in the peripheral field of skin in the neck and over the face.

Concerning the causation of the Brown-Séquard phenomenon, it might be argued either, on the one hand, that the raised excitability which conditions it is caused by over-stimulation of the afferent part of the mechanism by the degenerative changes in the skin area of the neck, and that these are due to the absence of proper grooming in virtue of the paralysis of the leg and foot and the loss of the scratching claws; or, on the other hand, it might be supposed that the section of the nerve produces a central change, one feature of which is a raised excitability of the scratch-reflex, and that the degenerative changes in the skin of the neck are conditioned either directly or indirectly by this central change and not by the peripheral paralysis and loss of toes.

The first theory is probably not correct. The Brown-Séquard phenomenon may be present when there is no visible degenerative change in the skin area of the neck and face. It may also be present when the scratching toes are not lost, and when the "voluntary" scratching movements are actually greater than usual upon that side. "Voluntary" scratching may even be so exaggerated and so effectual as to produce a bare patch of skin such as that mentioned above.

The second view must be held. Section of the great sciatic nerve actually raises the excitability of the scratch-reflex, and the degenerative changes in the neck area are due, not to excess of grooming, but to a diminution of grooming over the greater part of the area. This seeming paradox may be explained as follows:—The scratch-reflex varies as it is evoked from different areas of the total receptive field. Each of these various reactions is really, as Sherrington points out (44), an individual scratch-reflex different from all others. The section of the great sciatic nerve may be supposed to raise the reflex excitability for only a small group of these. That this is so is demonstrated by the restricted area from which the Brown-Séquard reaction may at first be obtained (45). When a "normal" stimulus—tickling caused by lice—occurs in the neck area, the scratch-reflex will be such that the scratching toes are directed to the point stimulated, and the reaction is an effective grooming of the skin. But if the excitability of a neighbouring group of arcs is raised by section of the sciatic nerve, then the normal response thus engendered may rapidly become one directed by irradiation towards the most excitable area. There will, in consequence, be a diminished normal grooming of the skin in all other areas. This will lead to an accumulation of lice, etc., and this condition may help to augment the raised excitability of the scratch-reflex.

But in what manner may the section of the great sciatic nerve produce the primary raised excitability of the scratch-reflex?

The author has previously described a conception of "neural balance" (59, 60). In general, it may be supposed that the activity and the reflex excitability of a centre—or of a pair of antagonistic centres—go hand in hand, and that they are the resultant of innumerable excitatory and inhibitory influences which continually play upon the centre. This resultant may be termed the "neural balance." The neural balance may be tilted for a time in one direction by the temporary preponderance of one of the influences which play upon it. And it may be permanently upset by a permanent alteration in the value of the influences which compose the balance.

With regard to the scratch-reflex, it has already been shewn (53) that removal of the cerebral cortex of one side produces such a permanent alteration. It is probable that section of the great sciatic nerve does also.

That the removal of so great a sensory field as that subtended by the sciatic nerve should increase reflex excitability in certain definite directions is likely. It is by no means improbable that the activity of the afferent mechanisms contained therein exerts an inhibitory influence upon the scratch-reflex; and that the section of the nerve permanently removes this inhibition.

In this connection it is interesting to refer to the observations of some other investigators. For instance, Langendorff (30) found that Goltz's croak-reflex—which may be evoked in the frog with regularity after decerebration—may also be evocable thus after section of the optic tracts alone. The removal of a peripheral sensory field here makes a specific reflex more excitable. He points out, too, that the raised excitability of the hind limbs, which is produced by packing the back of a frog in ice and was observed by Freusberg (28), may be due to the abolition in this manner of a large sensory field in the skin.

Of particular interest are the observations of v. Bötticher (31). He repeated Langendorff's experiments, and found, in addition, that section of both great sciatic nerves admitted of the regular evocation of the croak-reflex. And Mayer (33) found that the spinal cord was especially excitable on the injured side after section of one great sciatic nerve.

If the afferent impulses normally carried to the spinal cord through the great sciatic nerve tend in their activity to depress the excitability of the scratch-reflex, the causation of the Brown-Séquard phenomenon by the section of a great sciatic nerve resolves itself into the raising of the excitability of the scratch-reflex by the removal of an inhibitory influence. The fact that the phenomenon does not at once appear on section of the nerve may be explained by a condition analogous to spinal shock.

### V. *Experiments on Transmission of the Phenomenon.*

(a) *Experiments on Guinea-pigs.*—In the present experiments 29 young guinea-pigs have been born of 11 female guinea-pigs after removal of a part of the mother's right great sciatic nerve. Some of these were conceived by normal fathers before the operation. In the case of the others, both parents had their sciatic nerves cut. As in no case any congenital abnormality of the hind limbs was observed it is unnecessary to give further details.

Of these young guinea-pigs only five survived birth. One of the five was born within a short period of the operation, and before the mother had exhibited the phenomenon. The others were born in the third month after the operation, and when their parents exhibited the phenomenon. In none was there any malformation of the feet or toes, and there was no evidence of the presence of the phenomenon, although they were tested over periods of several weeks (the one which was born soon after the operation lived only two days, the other four lived for 7, 7, 26 and 28 weeks respectively).

Of the remaining 24 guinea-pigs all were dead at birth, or died within a few hours of birth; five were born prematurely; five were not damaged by the mother; the remaining 14 were all partially eaten. In some cases they were almost entirely devoured, in others scarcely touched.

No part of the body was exempt, although the parts usually damaged appeared to be the abdomen and limbs. In several cases the limbs were undamaged, but in four cases the hind limbs alone were injured.

These four instances bear on the present problem. In two cases the hind leg was eaten. In one case, one foot was eaten and nothing else; and, in the fourth case, only one toe of one hind limb was devoured. Had this last guinea-pig survived, and not been examined until the toe had healed, the malformation might well have been regarded as congenital.

(b) *Experiments on Rats.*—A portion of the right great sciatic nerve has been removed from 12 rats. Some of these were white rats, and others (five in number) were brown rats stated to be bred from a white tame mother and a brown wild father.

It was found that degenerative changes in the foot were far rarer in the rats than in the guinea-pigs from which a piece of the great sciatic nerve had been removed.

A "complete" phenomenon, similar to that seen in guinea-pigs, was not obtained. But it was found that the ordinary scratch-reflex—analogous to the "incomplete" phenomenon—was raised in excitability upon the side of the lesion. If the inside of the ear, or the skin behind the ear, was tickled, it was found that upon the side of the lesion the scratch-reflex was evoked, but this did not occur upon the other side.

These rats produced many litters of young. An exact register was not kept, but the number of young was greater than 120. Many of these (about 40 to 50, or more) were carefully examined, but a raised excitability to the scratch-reflex was in no instance seen. That is to say, that scratching was not obtained in response to a stimulus of equal value to that which produced, upon the side of the lesion, scratching in the parents.

## VI. *Conclusions Concerning the Alleged Transmission of the Brown-Séquard Phenomenon.*

If the preceding conclusions regarding the nature and causation of the phenomenon be accepted, they throw some light upon the question of the value of the experiments of Brown-Séquard as an example of the transmission of an acquired character, for the state itself cannot be regarded as an acquirement. The mechanism is present in every guinea-pig, and the possibility that it be rendered apparent in the phenomenon is always there. The peculiarity of the condition in the parent is that a certain specific mechanism in the central nervous system is rendered more excitable than usual. Thus the question of transmission in this case is not "is a mechanism which arises *de novo* in the parent transmitted to the offspring?" but "does a state of the raised excitability of a reflex mechanism already present in the parent condition by inheritance a similar raised excitability in the offspring?"

Before considering the answer to this question notice may briefly be taken of Galton's suggestion (27) that the appearance of the phenomenon in the offspring is due to imitation of the parent. This is, to say the least, extremely unlikely. In no case was such imitation seen in these experiments.

In support of the affirmative answer to the question the experiments of Brown-Séquard and of others may be quoted. If the negative be believed to be the proper answer, these results must be explained in some other way.

In regard to this question Brown-Séquard's experiments may briefly be summarised by stating that he found, after section of the great sciatic nerve in normal guinea-pigs, the appearance of a peculiar state termed by him "epileptic"; that this appeared in every instance, or in almost all instances, and that in most cases the toes were lost through the animals nibbling away the parts rendered anæsthetic. Of the offspring of such animals he found that a very small proportion exhibited a malformation of the feet, and that of these some exhibited the "epilepsy." The proportion of offspring which exhibited the "epilepsy" was only 1 to 2 per cent.

In connection with the congenital absence of toes in these offspring Morgan (40), in a criticism of the experiments, notes the possibility that the malformation may be due to a perverted act on the part of the mother at birth. He states that he had observed an absence of the tails in succeeding litters of white mice, and that this was due to the action of the mother. The mother bit the tails off and devoured them. This is not difficult to explain, for it probably is a perversion of the normal act whereby the mother devours the placenta and nibbles off the umbilical cord of her young at birth.

The observations described in this paper, in which actual injury to the toes of the young at birth was seen in the case of offspring of guinea-pigs in which the Brown-Séquard phenomenon was present, strongly support this view of the origin of the "congenital" malformations of the toes described by Brown-Séquard.

But this explanation may also serve to elucidate the nature of the "transmission" of the phenomenon itself. The observation described above, of a guinea-pig which developed the phenomenon in consequence of an accidental injury to its foot, demonstrates that the condition may be caused by such an accidental injury as that which may be inflicted by the mother upon its young at birth.

The experiments of Maciesza and Wrzosek (56, 57, 58) are of some interest. Their conclusion, that in the offspring of guinea-pigs in which the phenomenon is present section of the great sciatic nerve is followed by the appearance of the phenomenon at a smaller interval of time than is usual, requires some explanation.

But their results should be received with some hesitation until statistics from a larger number of experiments are available. The figures upon which they base their conclusions are obtained from 27 normal guinea-pigs and 14 offspring only. They take as a test the interval which elapses between the time at which the great sciatic nerve is cut and that at which the first appearance of the Brown-Séquard phenomenon is observed. This interval is found to be less on the average in the offspring of guinea-pigs which have the phenomenon in consequence of section of the great sciatic nerve than it is in normal guinea-pigs. Of the 14 offspring upon which their results are based one exhibited the "complete" phenomenon as early as the fifth day after section of the nerve, and another as late as the sixty-second day. With so great a variation no great value can be placed upon so small a number of results as 14. For instance, had they obtained two additional offspring, each of which exhibited the "complete" phenomenon upon the sixty-second day, the average for the whole would nearly have approached that which they obtained in the case of normal guinea-pigs. An additional criticism may be directed to their results in that they compare an average obtained from normal guinea-pigs with one obtained from the offspring of guinea-pigs which exhibited the phenomenon, but do not compare the latter average with that of their individual parents.

Even if it can be demonstrated without doubt that the offspring of "epileptic" parents on an average exhibit the phenomena within a shorter duration of time after section of the great sciatic nerve than did their parents, this would not necessarily prove that the state of raised excitability of the scratch-reflex is inherited.

The experience of the present author, and of Taft, and of Maciesza and Wrzosek, has shown that guinea-pigs which exhibit the phenomenon are unfitted to bear healthy young. Many of their young are dead at birth, many are aborted, many die shortly after birth. Those which survive are often less healthy than are the young of normal animals. One of the signs of this ill-health may be the absence of an efficient grooming of the skin. Lice may be more numerous upon them in consequence of this, and perhaps also in consequence of a greater direct infection from the mother than usual. This in itself will raise the excitability of the scratch-reflex. In this connection Prof. Sherrington has called my attention to the fact that "dirty" cats are more likely to exhibit the scratch-reflex after decapitation than are well kept ones. In a similar manner, it might be supposed that the young of "epileptic" guinea-pigs would exhibit the phenomenon within a shorter duration of time after section of the great sciatic nerve than would the offspring of healthy individuals.

## VII. *Summary.*

1. The Brown-Séquard phenomenon ("experimental epilepsy" in guinea-pigs) is nothing more or less than a specific instance of the

scratch-reflex. The "incomplete" reaction resembles the true scratch-reflex. The "complete" reaction resembles to a certain extent the "narcosis scratch" described by the author.

2. The true scratch-reflex may be evoked in normal guinea-pigs, and so may be the scratching phenomena of the narcosis scratch.

3. The Brown-Séquard phenomenon is due to a raised excitability of the scratch-reflex.

4. What especially is acquired as a consequence, for instance, of the removal of part of one great sciatic nerve, is a state of raised excitability of the mechanism which subserves the scratch-reflex.

5. The question of the alleged transmission of this phenomenon to the young of animals in which it is present therefore resolves itself into the question of the transmission of an acquired state of raised reflex excitability of the scratch-reflex.

6. Experiments here described prove that the state of raised excitability of the scratch-reflex in the parent is not due to the continued irritation caused by the formation of a cicatrix round the stump of the divided nerve. For division of the nerve again above the stump does not abolish the phenomenon.

7. Experiments also shew that the condition has no fixed relationship to the presence or to the absence of degenerative changes which sometimes occur in the foot after severance of the nerve.

8. Observations also shew that the phenomenon may occur in animals in which there is no "trophic" change in that area of the skin of the face and neck ("epileptogenous zone" of Brown-Séquard) from which the reaction is evocable by the application of mechanical pressure. The phenomenon may also occur when such a change is present there. This change is therefore probably not the intrinsic cause of the condition.

9. The suggestion is put forward that the raised excitability of the scratch-reflex, which conditions the phenomenon, is due to the removal of an inhibitory influence normally exerted by the great sciatic nerve and its branches.

10. It may be supposed that, in the "neural balance" of the scratch-reflex, one of the inhibitory factors is conditioned by the activity of the afferent fibres contained in the great sciatic nerve, and that, when this factor is removed by division of the nerve, the excitatory factors are less completely balanced, and the neural balance is tilted in the direction of excitability.

11. The raised excitability of a certain number only of the individual reflex arcs, which together compose the scratch-reflex, may lead to a state of incoördination in all the other arcs, so that, when a normal stimulus tends

to produce a reaction directed to the area of skin in which the stimulus is present, the reaction rapidly irradiates into the more excitable arcs. Thus efficient grooming of the skin will be prevented. Degenerative changes may then appear in the "epileptogenous zone," and these may tend to raise still further the excitability of the reflex by acting upon the excitation side of the neural balance.

12. If this is the nature and causation of the Brown-Séquard phenomenon, it is, at any rate, very difficult to see how it is transmitted to the offspring.

13. Experiments are quoted in examination of the alleged transmission of the phenomenon to the offspring in guinea-pigs and in rats—the presence of the "incomplete" phenomenon being shown for the rat.

14. As regards a state of raised excitability of the scratch-reflex in the young which survived these are negative. But emphasis is not placed upon these merely negative results, especially as they are comparatively small in number.

15. Of peculiar significance are three observations. In the first place, guinea-pigs which had a "trophic" change in the foot, as a result of the severance of the great sciatic nerve, have been seen repeatedly to nibble the feet of other guinea-pigs in the same cage which also had this change in the foot from the same causes.

16. The second observation is that accidental injury to the toes in a normal animal may be followed by the appearance of the Brown-Séquard phenomenon.

17. The third observation is that, in several instances, the young of guinea-pigs which had the phenomenon present have been noticed to have one or more toes eaten off by the mother. These young were probably alive at birth, but were dead shortly after. In some cases injury to the toe or to the foot was the only mutilation produced by the parent.

18. These three observations—that the phenomenon may be produced by accidental injury to the toes, that the parents evidence an abnormal habit of nibbling not only their own feet (in the anæsthetic parts) but also the feet of other similar guinea-pigs, and that the guinea-pigs may nibble the feet of their young—seem almost to prove a suggestion which has been put forward by Morgan, and seems to be hinted at by Taft.

19. It may be admitted that Brown-Séquard and others have actually seen the presence of the phenomenon in the offspring of these guinea-pigs. *But it may be supposed with every degree of probability that this was due to accidental injuries inflicted upon the young by their parents.* The statement

of Brown-Séquard that there were always malformations of the toes in these cases supports this view.

20. From direct observation, and from a consideration of the high rate of mortality, it may be supposed that the young of guinea-pigs which shew the phenomenon are less healthy than are normal guinea-pigs. There may then be a less efficient grooming of the skin, and, in consequence, the excitability of the scratch-reflex will be raised, as in other animals. This makes of little value evidence that the phenomenon appears sooner after division of the nerve in the young of guinea-pigs which already have the phenomenon than in the young of normal guinea-pigs, even if that evidence be definitely established.

21. We may conclude by saying :—

An examination of the "Brown-Séquard phenomenon" in guinea-pigs—usually considered to be a classical instance of the alleged transmission of an acquired character—throws much doubt upon its value in this controversy.

The phenomenon is not an acquired peculiarity produced *de novo* on division of a great sciatic nerve. It is due to the raised excitability of a mechanism—that of the scratch-reflex—already present; and this raised excitability is probably due to the removal of an inhibiting influence by section of the nerve.

The phenomenon, therefore, cannot be considered as transmissible as an acquirement *per se.* If anything is transmitted as an acquired character it must be the state of raised excitability of the scratch-reflex.

The presence of the phenomenon in the offspring observed by Brown-Séquard may be admitted, but this may be explained otherwise than by assuming a transmission of acquired characteristics.

That the alternative explanation—the presence in the offspring is due to a production of the state by injury to the toes and feet inflicted by the parent—is true is rendered possible, and indeed highly probable, by certain parallel evidence submitted in this paper.

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# *Note on* Astrosclera willeyana *Lister.*

By R. Kirkpatrick.

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As the result of an investigation of 47 specimens of *Astrosclera willeyana* dredged by me off Christmas Island, Indian Ocean, I have found that this organism is a Siliceous Ectyonine Sponge with a supplementary skeleton of aragonite. The sponge owes its unique character to the fact of its being associated with a degenerate Floridean Alga. Sponge cells capture and envelop the algal tetraspores and carpospores and secrete around them concentric layers of aragonite. The spherules so formed are in many respects comparable with the cyst-pearls of Mollusca. Just as certain Ectyonine sponges make supplementary skeletons out of foreign particles of sand, Foraminifera, etc., so Astrosclera builds a similar kind of skeleton out of the spherules. The alga probably comes under the Ceramiales Oltm., and appears to belong to a new genus and species, of which a provisional diagnosis is given below :—

*Rhododiplobia*,* n. gen., Alga degenerata, in spongia (scilicet *Astrosclera willeyana* Listeri) symbiotica, partim in carne, partim in calcis sceleto duro sita.

Thalli plantarum sexualium minimi, et in spongiæ carne viventes.

Thallus ♀ carpogonia binis trichophoris et trichogynis ornata, thallus ♂ antheridia ramosa gerens.

Thallus asexualis in calcis sceleto duro perterebrans simulque in carne vivens, filiformis, monosiphonius, septatus (raré articulatus), ramosus; stichidia, tetrasporangia cruciatis tetrasporis præbita, gerens.

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* ῥοδό (in comp.), red (alga) ; διπλόος, double ; βίος, life ; referring to the habitat in the solid calcareous skeleton, and in the soft tissues of the sponge.

Color, fere sine dubio, aurantiacus (sensu Saccardoï).

*R. cor-margaritae,*[*] n. sp., Diagnosis ut genus.

A fully illustrated account of the very remarkable life-history of *Astrosclera-Rhododiplobia* will shortly be published, which, I trust, will furnish clear proof as to the accuracy of the statements here made. The two organisms have become, in all reasonable probability, indispensable to each other and inseparable. The sponge embryos, before they have left the parent, are associated with plants bearing carpogonia antheridia or stichidia, and even the youngest sponges, barely visible to the naked eye, have solid well-built skeletal walls composed of aragonite spherules each with an algal spore in its centre. I consider that the association has become one of true symbiosis, and is not merely an instance of parasitism of the alga on the sponge, or *vice versâ*.

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[*] *Cor*, heart; *margarita*, pearl.

## ERRATUM.

Page 141, lines 4 and 6 (from bottom), and p. 142, 2nd column of table, *for* postometer *read* potometer.

# OBITUARY NOTICES

OF

# FELLOWS DECEASED.

# CONTENTS

PAGE

Sydney Ringer. (With portrait) ........ i

Sir Rubert Boyce ........ iii

Sir Francis Galton ........ x

John Hughlings Jackson ........ xviii

John Beddoe ........ xxv

Sydney Ringer

## SYDNEY RINGER, 1835—1910.

SYDNEY RINGER, who died at Lastingham, in Yorkshire, on October 14, 1910, was the son of John and Harriet Ringer, of Norwich, where he was born in 1835. He was educated at private schools, and at the age of 19 entered, as a medical student, University College, London, with which institution he was to remain connected during the remainder of his active life. At the hospital connected with that school he was successively House Physician, Resident Medical Officer (1861), Assistant Physician (1863), full Physician (1866), and Consulting Physician (on his retirement in 1900); and in the Faculty of Medicine of University College he held successively the chairs of Materia Medica and Therapeutics, of Medicine and of Clinical Medicine. The School of Medicine with which Ringer was associated has produced many distinguished clinicists, but it may be safely affirmed that it has produced no better clinical teacher than the subject of this memoir. It was not, however, on the ground of his clinical reputation that Ringer was elected a Fellow of the Royal Society, and it is not in the notices of this Society that his eminence as a clinicist need be accentuated. For Ringer was more than a great physician, much as that may mean: he was a scientific enquirer. His bent in that direction showed itself early, for even while still a student of medicine he presented a paper to the Royal Society, "On the Alteration of the Pitch of Sound by Conduction through different Media," and others to the Royal Medical and Chirurgical Society on Metabolism in Disease. These were followed by an investigation (conducted jointly with A. P. Stuart) into the diurnal variations of temperature in the human body, which was, however, not published in full until 1878. The subject of this enquiry, from its bearing on the variations of temperature in fever, never lost interest for him. But his appointment to the chair of Materia Medica and Therapeutics directed his attention towards the action of medicinal substances and agencies. His experiences of their action on the human body he embodied in his well-known 'Handbook of Therapeutics,' of which a very large number of editions have appeared; no more thoroughly practical handbook of treatment has probably ever been written. Ringer, however, recognised that it is necessary for the understanding of the action of remedies in disease for their action in health first to be determined, and that, to comprehend their effects upon the body generally, their influence upon the individual organs and tissues must be understood. There was then no laboratory of pharmacology in London, but he found the opportunity for carrying out researches of this nature in the Physiological Laboratory of University College, where a place was always at his disposal. Here, in the intervals of a busy consulting practice, he carried out the remarkable series of researches on the action of various salts upon the tissues, and especially upon the muscular tissue of the heart, which resulted in the recognition of the influence exerted by simple inorganic constituents of the blood in maintaining the activity of the living tissues—an influence which had remained obscure, in spite of the elaborate series of researches of the same nature which were conducted in the famous Physiological Laboratory of Leipzig and elsewhere.

Ringer was the first to show that a solution containing certain ions (chlorine, sodium, calcium, and potassium), in the form of inorganic salts in definite proportions, provides a fluid which can completely replace the ordinary blood of an animal in so far as the activation of the living tissues is concerned, and that the presence of these ions or others of similar nature is necessary for such activation. Such a fluid is now in general use in physiological laboratories and is known as "Ringer's solution."

Later he extended these researches to embrace the action of the same salts upon the heat-coagulation of proteins and upon ferment-actions such as those producing the clotting of blood and the curdling of milk. Above all he was instrumental in discovering the important part which calcium plays in most of these processes. He also carried out numerous investigations into the action of special drugs, such as veratrine, muscarine, pilocarpine, and aconitine, and was the first to investigate the direct action of anæsthetics upon cardiac tissue. Some of these researches were conducted with the aid of fellow-workers, many of whom have since obtained distinction in the medical profession.

Ringer was elected a Fellow of the Royal Society in 1885.

His methods were of the simplest and were but little varied. For registering the effects of salts and drugs upon the heart he employed Roy's tonometer; their effects on skeletal muscle were recorded by the ordinary student's myograph; their effects on blood-vessels by adding some of the drug to the fluid employed for perfusion and counting the drops which passed through the vessels in a given time. He used for these investigations the tissues of the frog, rarely, if ever, employing mammals. Even if he had desired to carry out experiments on the higher animals, it would have been difficult for him to find enough time. His scientific work was done between breakfast and the commencement of his private practice, which could give him at most a couple of hours a day; this was sometimes supplemented by a visit to the laboratory in the late afternoon. Clinical medicine was his profession, scientific research was his recreation. As he himself would have been the first to admit, in science he was an amateur. But, we may justly add, the sort of amateur who produces better work than that of many a professional!

His period of greatest activity is contained between the years 1875 to 1895. During this time he published—for the most part in the 'Journal of Physiology'—a succession of papers on the various subjects which have been above indicated.

No notice of Sydney Ringer would be complete without reference to the personal qualities which characterised him. His upright carriage, open, frank countenance, and animated movements found their counterparts in mental characteristics which were equally typical. In disposition he was the most modest of men, and it was with difficulty that he was induced to allow his name to be proposed for the Fellowship of the Royal Society, although his friends were well aware that his selection would follow as a matter of course.

Although holding decided views on social and religious questions, he never allowed them to be obtrusive. The generosity of his nature and the kindliness of his disposition were exemplified in many ways, and in numerous instances the persons whom he assisted never knew the name of their benefactor.

He is laid to rest in the churchyard of Lastingham, at the edge of the Yorkshire moors, by the side of his beloved wife and of a daughter, early lost to them, in remembrance of whom her parents restored the beautiful old village church. His memory is cherished by his friends and honoured by physiologists throughout the world.

E. A. S.

## SIR RUBERT BOYCE (1863—1911).

THE death of Sir Rubert Boyce in June last at the age of forty-eight came as a shock to many. He was born on April 22, 1863, in London, and London was his early home, but his parentage was Irish. His father, Robert Henry Boyce, of Carlow, was an engineer, at one time Principal Surveyor of H.M. Diplomatic and Consular Buildings in China. His mother was a daughter of Dr. Neligan, a medical practitioner of eminence, in Athlone. Boyce's trend toward natural science began early. Sent to a preparatory school at Rugby, he there acquired a practical knowledge of botany, amplified during his holidays in London by microscopic work with his parents' friend Mr. Hurst, a member of the Quekett Microscopical Club and author of a handbook on surveying. Later he was at school at Paris, where his aunt, Miss Henrietta Boyce, was then resident. It seems that during his boyhood he picked up knowledge of several handicrafts—carpentry, mason's work, plumber's and glazier's fitting. To these latter he would turn on occasion in after years as the nearest things to recreation not ennuyant to him.

He entered on the study of medicine, his place of studentship being University College, London. In 1888 he obtained the diplomas of the Royal

Colleges of Physicians and Surgeons, and in the following year the degree of M.B. of the University of London. He never proceeded to the full doctorate. He was not one who attached much weight to formal examination results. Moreover, in later years he would go out of his way to tell friends that the University system in force in the metropolis in his day had never given him an *alma mater.*

After obtaining his degree he became an assistant in the Pathological Laboratory under Professor Victor Horsley, at University College. There his energy and ability soon showed. In 1892 he was appointed Assistant-Professor of Pathology. He contributed conspicuously to the large output of research from the laboratory, and he issued a text-book of Morbid Histology, a volume of 400 pages. The book was never very popular with students. It was probably too original for them. Its preface stated that in it "little stress was laid upon the ordinary methods of classification"; it was also full of excellent microphotographs, a class of illustration then novel of adoption for such a purpose.

In 1894 Boyce was appointed to the newly-endowed Chair of Pathology in the young University College of Liverpool. He threw himself at once into the task of organising a laboratory of scientific Pathology on modern lines. His laboratory quickly became a centre for workers attracted by and sharing his enthusiasm. Much valuable research issued from it. Greatly though his laboratory absorbed him and flourished, problems concerning the University College as a whole began to occupy him even as much or more. On the College Senate he became a force urging towards development and expansion. His activity in this direction soon passed beyond the immediate circle of the Senate and its routine business. He embraced every opportunity, public or private, to make his voice heard as a preacher of ampler University activity. It was soon evident that he could make others, even those engaged in pursuits seemingly alien and remote from his own, listen; he won their sympathy and support. An early success he achieved may be cited as illustrating his character and policy. In his view the College was *de facto* a University; he also realised that an immensely increased sphere for public preventive medicine was at hand. He urged it as the duty of, and opportunity for, the College to take up vigorously forthwith the teaching of hygiene, technically, practically, and yet scientifically, to all in the community entering on its practice, even in its humbler aspects—sanitary inspectors, meat inspectors, builders, and plumbers. To the academic body this did not greatly appeal; its apathy chilled Boyce little. Unsupported, he went outside to laymen; to them he presented a scheme with convincing capacity and persuasiveness. Almost at once he obtained the gift of two houses adjoining the University College, their remodelling and equipment as a laboratory and museum, and a subvention for their maintenance as such. The Lord Mayor opened the School of Hygiene formally, and the University College itself looked on with surprise at its own enrichment and the expansion of its scope. This, Boyce's first appearance as a local public force, was significant of much in his further career. It revealed his boldness and shrewdness of appeal for University aims to a non-University public, and his ideal of a University life dovetailed by public utility into the life of a civic community.

Looking back to that time, we know now that a wave of University development was then, in fact, imminent in the country. And we know that Liverpool proved one of the chief centres of its motive force; in that centre Boyce was eminent as a forceful and practical spirit. In Liverpool the problem naturally presented itself particularly as that of enlarging and freeing the University College to a fully-equipped and self-centred University. The College at Liverpool, together with similar colleges in Manchester and Leeds, was nominally centred outside Liverpool at Manchester. This conglomeration Boyce felt should be broken up. At Liverpool within the academic body itself diffidence opposed such a departure. In the outside community indifference and want of appreciation of the issue had to be removed. Caution urged "let well alone." Many even among those best disposed toward University projects feared that a large demand for further funds would fail or would deplete schemes already working and requiring steady upkeep. They thought that to undertake such wide new responsibilities would bring inability to meet adequately either the new or the old. To all such fears Boyce's courage was deaf. His answer came less in words than in deeds. His energy and resource left no stone unturned in search for ways and means. Allying himself with a few colleagues, styled intimately "The New Testament," and chiefly of the Arts faculty, he with them started a University Club. Its housing and cuisine were almost ostentatiously Spartan, contrasting against the luxurious clubs of the commercial city. Its means at outset were of the most slender. Boyce's contributions were not the less valuable because they extended even to the house furnishing; as a capable bricklayer he built with his own hands a wall in the club yard. This club achieved its aim. Formed to consolidate the local University movement by bringing into close social relation men from inside and from outside the College circle itself, it became the rallying point for those ventures which culminated in the formation of the present University. Boyce was president of the club in one of its most eventful years.

In 1898 the Department of Pathology entered into occupancy of a fine building erected and equipped for it by the late Rev. S. A. Thompson-Yates. Almost at the same time Boyce was appointed bacteriologist to the Liverpool Corporation. The opportunities the new laboratory and the new post together opened to him were just such as his heart desired. The work particularly interested him; moreover, he saw himself and his laboratory serving as a substantial bond between the University College he so cherished and his adopted city of which he was so proud. In daily touch with the Municipality and the life of commerce and its leaders, he made friendships of lifelong endurance, and became conversant with ways and views novel to his experience. When in 1902 the movement for establishment of the University took final shape, his influence contributed with unique effect.

In a collective enterprise, where action and action interact, it is difficult to assign to individuals their respective measures of effect. But it is certain that to Boyce, as much as to any one person, the University movement in Liverpool owed success. After the actual institution of the University his labours for it still continued, multiplying rather than abating. Four endowed Chairs have owed creation largely to him, the Chairs of Bio-Chemistry, of Tropical Medicine, of Comparative Pathology, and of Medical Entomology, as well as the University Lectureship on Tropical Medicine.

In the meanwhile his position and experience as a bacteriologist led to his engagement on work of national scope. He was appointed a member of the Royal Commission on Sewage Disposal. Much of the research executed for this Commission was done in his laboratory, with the assistance of Dr. (now Professor) A. S. F. Grünbaum and Drs. Harriette Chick, Hill, and MacConkey. Later, in 1904, he became a member of the Royal Commission on Tuberculosis. On the day of his death he was to have given his signature to the final Report of that Commission.

In 1897 Boyce visited Canada with the British Association. He was a secretary to the section of Physiology. The meeting was at Toronto. This visit made a lasting impression on him. Closer union of the Dominion with the old country by ties of mutual help and understanding became with him a cherished ideal, and, as usual, he was not idle in regard to it. By his advice, Mr. William Johnston, of Liverpool, instituted a Fellowship in the University for young medical graduates from parts of the Empire outside the Three Kingdoms. The steady success of the occupants of this Fellowship, coming into the University from Canada and elsewhere, was an abiding pleasure to Boyce in all his after years.

His ardour for Imperial development found congenial application later when a letter reached the Faculty of Medicine from Mr. Chamberlain, then Colonial Secretary. The letter rehearsed the heavy toll on life and health taken by trade with the Tropics, a trade with which Liverpool as a port is deeply concerned. The letter urged that the School of Medicine at Liverpool might well establish a department devoted to the special study of tropical disease. It is no secret that at first the suggestion was not well received by the Faculty. Some regarded it as a rather presumptuous piece of official interference: already, a whole hour's lecture in the systematic course on medicine was entirely devoted to malaria. But Boyce's mind caught fire from the new proposal. He would do it himself if the Faculty would not. He would set apart rooms of his own, and, if need be, himself raise the money necessary. And on the task he embarked at once with his habitual energy. A public dinner in connection with the Royal Southern Hospital took place a little later. Boyce spoke to one of the toasts, and took opportunity to plead for the new cause. Sir (then Mr.) Alfred Jones was present. Sir Alfred used to relate with relish "before that dinner was over Boyce had a hundred pounds out of me." Co-operation thus began between two men of somewhat similar energy and kindred imagination. Their alliance tightened and strengthened. It was broken only by Sir Alfred's untimely death in 1909. By them in conjunction was founded the Liverpool School of Tropical Medicine, now famous the world over. They launched its pioneer work of combatting the diseases of the Tropics. Boyce organised the scientific and technical part of the scheme; he also collected a large part of the funds. In a country where there are few or no governmental subventions the only course open is the familiar way of all the public charities. Boyce sometimes told his friends that when he died the word "cash" would be found written across his heart. But his indefatigable hunt for funds was pursued with considerable sense of humour. It often became a game wherein no one was more amused than the wealthy and generous man who, meaning to be close fisted, found he had subscribed handsomely. As time went on the care of the new school and consequently the exploration—one might almost say the exploitation—of tropical disease in general became the interest most absorbing Boyce. His history becomes largely a history of the school itself. An initial question had been the appointment of a Director. To the disappointment of sundry local hopes there was for Boyce's mind but one man possible, Major (now Professor Sir) Ronald Ross, then on his way home from India, discoverer of the mosquito-borne nature of malaria. Ross was secured, and the Directorship soon became, through Sir Alfred's generosity, an endowed University Chair. In 1901 commenced the series of expeditions sent by the School to tropical countries to investigate the diseases in their habitat there. In the first six years of its existence the School despatched no fewer than seventeen expeditions. Costly in life and money as these were, they were also rich in theoretical and practical results. Boyce pushed their prosecution with an unfailing optimism. In 1905 he himself went to the yellow fever outbreaks in New Orleans and British Honduras.

It was in September, 1906, that, in a period of strenuous work exceptional even for him, at Harrogate, where he wished to establish a sanatorium for patients from the tropics, Boyce was struck down by a paralytic seizure affecting his left side. He faced the disaster with a courage truly heroic. He never regained complete power in his arm and leg, but after twelve months he partially resumed work at the University. He evidenced some lack of emotional control, but his vivacity was unabated and his desire to be doing just as keen as ever. Partially cut off from other work he devoted himself unsparingly to the campaign, by that time become international, for securing a cleaner health bill for the Tropics. Invalid though he was, he visited the West Indies to report at the instance of the Government on yellow fever in 1909. West Africa for the same purpose he visited in the following year. Not content with official reports of these expeditions he set to work to impress the importance of tropical preventive medicine on the general public. The result was the publication in two short years of the books 'Mosquito or Man' and 'Health Progress and Administration in the West Indies.' Written in a clear style and addressed to the general reader, these set forth the bearing of recent biological discoveries on human life and commercial prosperity in tropical communities. These books found an immediate sale. Of the former there have been three, of the latter two editions. In January of the present year he published a third volume, 'Yellow Fever and its Prevention'; this he dedicated to the late Sir Alfred Jones, "whose vivid imagination and great grasp of affairs stimulated the author to travel." Through these books and his other work Boyce's name, it is not too much to say, has become familiar to every European in the Tropics. The last completed of his projects was the formation, at Liverpool, of the Bureau of Yellow Fever. He finished the first number of its 'Bulletin,' and sent it to press only an hour before his final seizure.

On May 2 of the present year, while on his way to attend a meeting in London of the African Advisory Board, he was attacked with motor aphasia and slight paralysis of the right side. He returned to Liverpool, and, in the course of a few weeks, made considerable recovery. So soon as he felt better, no arguments could induce him to rest or forego his public calls. On June 7 he attended a banquet of the Tropical School held to welcome back his old friend Prof. Todd of Montreal and the other members of the Gambia Expedition, and to wish good-bye to Prof. Newstead, then starting for Uganda. Boyce responded to the toast of "Tropical Medicine and Commerce." A week later he had an apoplectic seizure; he lost and never regained consciousness; on the 16th he died.

He had married in 1901 Kate Ethel Johnston, a daughter of Mr. William Johnston, shipowner, of Liverpool, a munificent benefactor to the University. The Tropical School is housed in laboratories given by Mr. Johnston and bearing his name. Boyce lost his wife a few days after the birth of their only child, a daughter.

The foregoing sketch will have indicated how much Sir Rubert Boyce accomplished in the brief span permitted him. In 1906 he was created a Knight Bachelor for his services to tropical medicine. In figure he was small, fair, light, and active. He took a lively interest in arts of decoration and design. His house contained interesting pieces of old furniture and a large collection of fine Persian tiles. He entertained with wide hospitality friends and visitors from all parts of the world.

Strenuous, impetuous, sometimes intolerant of opposition, he had tact, humour, and good nature as well as decision and shrewdness. His views were bold and imaginative. Constantly obliged to work through committees, he always remained somewhat rebellant against the delays inherent to that system and procedure. Many of his most valuable and farthest reaching steps on behalf of his University and the Tropical School were taken and their business almost completed before his Committee had become formally aware that he had moved. His methods frequently came as electric shocks to those accustomed to ways more sedate. Financial obstacles seemed to present no difficulty to him where he felt an aim desirable. His activity not rarely exposed him to keen antagonism. He met this with various moods, but it never troubled him much. He won with curious facility the sympathy and confidence of men of business and affairs. He made the pursuit of science intelligible to them in the same way as it was to himself. In council with actual men of science he was less effective. His gifts appealed to them less; his little weaknesses were of a kind particularly evident to them. In his earlier years he gave much promise as an investigator in scientific pathology. In 1902 the Royal Society elected him a Fellow. But he was already then too engrossed in organisation and administration to contribute much further to original research. His work for the expansion of his University and its School of Tropical Medicine absorbed him more and more. They precluded concentration of his mind on other problems. Embarked upon propagandism the temperament attaching to that shifted his mental key unsuitably for the prosecution of exact research. His own interests often suffered from his devotion to public business. His name should be remembered as an apostle preaching the importance of applied science successfully to the laity of his time. It will assuredly remain honoured in the University he so devotedly helped to raise; so also in that School of Tropical Medicine which grew from his inspiration. That School's success was the great aim and reward of all his later life. When the history of the university movement in England at close of last century and beginning of this comes to be written his should be a name of prominence in more than one of its pages. In any history of the development of tropical medicine his place as an organiser and a leader must be among the foremost in an epoch-making time. C. S. S. (September, 1911).

## SIR FRANCIS GALTON, 1822—1911.*

Sir Francis Galton, Knight, traveller, meteorologist, pioneer in the science of heredity, and founder of the school of "Eugenics," was born at Birmingham on February 16, 1822. He was the youngest member of a family of four daughters and three sons born to Samuel Tertius Galton (1783—1844) and his wife, Frances Anne Violetta (1783—1874), daughter by the second marriage of Dr. Erasmus Darwin (1731—1802), the philosophical poet and man of science. In recording the life of one who devoted himself so largely to the study of heredity (a word imported into the English language by Galton himself), it is natural to look to his ancestry as explanatory of his great intellectual powers. In every case of conspicuous ability such an inquiry might, indeed, be of interest, but it would frequently be impossible to attain any such degree of completeness as is possible in the present case.

The Galton family was probably originally settled at Galton, in Dorsetshire, and they were certainly inhabitants of Somersetshire in the seventeenth century, but the first to move to the neighbourhood of Birmingham was Francis Galton's great-grandfather. The family belonged to the Society of Friends, and, like many other Quakers, they were keen and active men of business. In their case the business was that of gunsmiths and ultimately of bankers, and in these pursuits considerable fortunes were amassed.

Many of the family, and of the Barclays with whom they intermarried, were remarkable men and women. Amongst those known beyond the local and family circles were Sir Ewen Cameron of Lochiel (1629—1719), Robert Barclay (1648—1690), the Quaker apologist; Galton's great-uncle, Robert Barclay Allardyce, better known as Captain Barclay (1779—1854), and celebrated for his great feats of endurance and strength; and his aunt, Mary Anne Schimmelpenninck (1778—1856), a well-known writer in her day.

On the maternal side, his mother was daughter of Dr. Erasmus Darwin, and he was therefore first cousin, of the half blood, to Charles Darwin, the well-known naturalist. His grandmother, the second wife of Dr. Erasmus Darwin, was the widow of Colonel Edward Chandos Pole, of Radbourn, Derbyshire. Her mother's name was Collier, and it may be asserted with some degree of confidence that she was a natural daughter of Charles Colyear, second Earl of Portmore (1700—1785), a member of a remarkable family.† It would be out of place to go into further detail here, but enough has been said to show that Galton's ancestry comprises more than a common allowance of remarkable men and women.

After attending at several small schools during his childhood, Galton was sent to King Edward's School at Birmingham. He describes his time there as a period of stagnation, for he had little taste for the purely classical teaching then customary, and had no opportunity of obtaining other kinds of instruction which he would have eagerly embraced. As it was intended that he should follow the medical profession he left school early, and after some preliminary apprenticeship to medical men in Birmingham he entered for a year's study at the medical school of King's College, London.

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* Sources—'Memories of my Life,' by Francis Galton (Methuen, 1908); personal knowledge, and private information. A life is being written by Prof. Karl Pearson, F.R.S.

† See article "Colyear, Sir David," 'Dict. Nat. Biog.'

In 1840 he made a rapid tour to Vienna, Constantinople, and Smyrna. Such a journey was not at that time nearly as easy as it is now, and it is only mentioned as indicating his early desire to travel off the beaten track. In October of the same year he entered Trinity College, Cambridge. At Cambridge he formed friendships with many men who afterwards became famous, and he considered his University career to have been of the greatest service to him in promoting his intellectual growth. He read mathematics with the celebrated tutor, William Hopkins, and he obviously had a considerable aptitude for that branch of study. However, a severe illness during his third year at Cambridge made it impossible for him to persevere with this course of reading, and he proceeded to take the Ordinary or "Poll" degree. Throughout his life he had a warm affection for his University, and amongst the honours which he appreciated most highly in later life was his election in 1902 to an honorary fellowship at Trinity College.

In 1844, just after Galton had taken his degree, his father died, and under the will he found himself in possession of means ample enough to permit him to abandon the contemplated medical profession and to give rein to his aspirations for travel.

Accordingly in 1845 he went up the Nile as far as Khartum and afterwards travelled in Syria. Such a journey was at that time an adventurous one, and it served in his case as an incentive to the exploration which he undertook some years later. On his return from the East he gave himself from 1845 to 1850 to the sporting pursuits of a country gentleman, but these amusements did not suffice to satisfy his ambition. He had become a member of the Royal Geographical Society, and had in that way made the acquaintance of many distinguished travellers. Fired by their example, he determined on making an exploratory journey at his own expense, and after considering for some time whither he should go, he fixed on Damaraland as the place of travel. Damaraland is now German territory and is fairly well known, but at that time it was completely unexplored. He started inland from Walfish Bay and penetrated far into the interior, meeting with many dangers and hardships on the way. An interesting account of this journey is contained in his work 'Tropical South Africa,' published in 1853, and the importance of his daring exploration was recognised by the award of medals by the English and French Geographical Societies.

It was in 1853, and thus not very long after his return, that he married Louisa Jane, daughter of George Butler, Dean of Peterborough and previously Headmaster of Harrow School. The marriage was a singularly happy one, but unfortunately they had no children. Mrs. Galton died at Royat in 1897, after a long period of ill-health. After her death one of her nephews lived with Galton for a time, and subsequently one of his own great-nieces was his companion up to his death.

After his African journey Galton was regarded as amongst the leading explorers of his time, and he played an important part in the work of the Royal Geographical Society during many years, indeed until increasing deafness prevented him from being a useful member of the Council. He was elected a Fellow of the Royal Society in 1856, and often served also on the Council of that body.

Whilst in Africa he had been struck by the waste of energy incurred by the fact that every explorer has to learn by bitter experience the numerous devices required for his safety and comfort, and he thought that much of this waste might be obviated if the experiences of travellers could be shortly set forth. He accordingly conceived the idea of collecting hints for travellers derived not only from his own experience in Africa, but also from that of others in widely different latitudes. The result was a small book published in 1855 entitled 'The Art of Travel.' It has since been through several editions and is a valuable *vade-mecum* for the explorer. It is much more than a dictionary of artifices to be employed in emergencies, and the present writer has found it very interesting reading.

After their marriage Mr. and Mrs. Galton settled in London, ultimately at 42, Rutland Gate, Hyde Park, and went much into Society, especially in literary and scientific circles. His powers as a conversationalist and ready humour, seconded by Mrs. Galton's sympathetic nature, rendered them charming hosts and they were universally popular.

The African journey had tried Galton's health severely, and he reluctantly felt himself compelled to forego further exploration, but he and his wife travelled extensively in Europe, and he became an enthusiastic mountaineer and member of the Alpine Club. There remains but little more to be recounted as to the social side of his life. He gradually became very deaf, and this cut him off much from the enjoyment of general society, but only in the last year of his life he learned of the existence of a microphonic form of ear-trumpet which restored his power of hearing to a marvellous extent and contributed greatly to his pleasure. During the last four or five years he became very infirm in body, although his intellect remained as bright as ever.

A portrait in water-colour, by O. Oakley, of Galton at the age of 22, and another in oil in later life by C. W. Furse, are in the possession of his nephew Edward Galton Wheler at Claverdon Leys, Warwick. A copy of the latter by F. W. Carter hangs in the Hall at Trinity College, Cambridge. There is a bronze bust of him dated about 1909, and executed by Sir George Frampton, at University College, London.

In 1908 he published an amusing and interesting account of his experiences entitled 'Memories of my Life,' which has served to furnish much of this present article. This work gives in an appendix a list of all his writings up to 1908.

He received many other recognitions of his scientific eminence by public bodies, besides those already mentioned. Thus in 1886 he was awarded by the Royal Society one of the annual Royal Medals; in 1891 he became Officier de l'Instruction Publique de France; in 1894 and 1895 he received the honorary doctorates of Oxford and of Cambridge; in 1901 and 1902 he received the Huxley Medal of the Anthropological Institute and the Darwin Medal of the Royal Society; in 1908 he was awarded the special medal of the Linnæan Society, struck to celebrate the fiftieth year since the presentation to that Society of the celebrated papers by Darwin and Wallace, which were the prelude to the publication of the 'Origin of Species.' Finally in 1910, only two months before his death, he received the highest award of the Royal Society, namely, the Copley Medal, but he was too infirm to receive it in person from the hands of the President. He received besides the honour of knighthood by patent on the occasion of the celebration of the birthday of King Edward VII in 1909. All these honours came to him very late in life, and the delay is to be attributed to the very originality of his researches, which did not fit easily into the numerous compartments into which scientific investigation has naturally come to be divided.

During his later years it was his habit to leave London during the winter, and he died of acute bronchitis on January 17, 1911, at Grayshott House, Haslemere, a house which he had taken for the winter months. He was buried on January 21, at Claverdon, near Warwick, in the family vault. His will contained some very remarkable provisions, which will become more intelligible when a sketch has been given of his scientific career.

Galton bore his full share in the administrative side of scientific enterprise. Thus from 1863 to 1867 he was the General Secretary of the British Association for the Advancement of Science, a body whose functions it is unnecessary to explain in these pages. It is well known that the success of that Society depends in a very great degree on the activity of the Secretary, and in his case the Council had made a good choice. Besides this he was four times a Sectional President, and twice he felt himself compelled to decline invitations to become President on account of his deafness and failing strength.

In 1863 Galton published an important book entitled 'Meteorographica, or Methods of Mapping the Weather.' It was already known at that time that storms consist of a "cyclonic" motion of the air round a region of low barometric pressure, and that the circulation is counter-clockwise in the northern hemisphere and clockwise in the south. In this work he pointed out that the interstices between cyclones are filled in by systems, to which he gave the name, now universally adopted, of "anticyclones," in which the circulation takes place round a region of high pressure and is clockwise in our hemisphere. He pointed out that the anticyclonic systems are of equal importance with the cyclones for an adequate apprehension of the causes of the variability of weather. He thus completed the basis of the system of weather forecasting which is now in operation over the civilised world. At a later date he also did much to formulate succinct methods of recording the multifarious results of meteorological observation.

This meteorological discovery doubtless explains how it came about that Galton was intimately associated with FitzRoy's early attempts to organise at the Board of Trade a meteorological service in this country, and it led to his membership from 1868 until 1900 of the Meteorological Committee (and of the subsequent Council), the governing body of the Meteorological Office. His position in meteorology had previously led to his association with the work of Kew Observatory, an institution initiated by General Sir Edward Sabine for magnetic and meteorological observation, and for the testing of instruments of precision. He was a member of the governing Committee from soon after its foundation, and Chairman from 1889 to 1901, in which year the Observatory became the nucleus of the National Physical Laboratory subsequently moved to Bushey. In this connection it may be mentioned that he did much to promote the efficiency of the institution, but we must refrain from going into details on this head.

But meteorology did not nearly suffice to occupy Galton's active mind, for already in 1865 he was occupied with those researches with which his name will always be associated. His investigations into the laws of heredity, to which we shall refer more in detail hereafter, led him to perceive the lamentable deficiency of tabulated data concerning human attributes. He therefore initiated an anthropometric laboratory at the International Health Exhibition of 1884. In this laboratory, statistics were collected as to the acuteness of the senses, the strength, weight, and dimensions of a large number of people. It might be tedious to recount all his work in devising instruments of measurement, in organisation, and in inducing others to work for him, and it may suffice to say that the outcome has been the collection of a mass of facts previously unattainable.

The impulse given through the collection of these anthropometric data, and afterwards by the publication in 1889 of his work 'Natural Inheritance,' gave the force which moved Weldon and Karl Pearson to undertake their far-reaching investigations. Thus the anthropometric laboratory at the Health Exhibition may be considered as the forerunner of the Biometric Laboratory subsequently founded at University College, London.

Amongst the data collected by Galton were impressions, made with printer's ink, of the fingers of a very large number of persons. It occurred then to Galton that such impressions might serve as a means of identification. Sir William Herschel had wished to use them for the identification of criminals in India, and Dr. Faulds had made a similar suggestion in this country, but there remained much laborious work for Galton to do. Proofs more decisive than any previously furnished had to be obtained that the finger-prints are permanent from youth to old age, that no two are exactly alike, and that the patterns are susceptible of arrangement according to types and classes in such a way as to render it possible to construct a dictionary of finger-prints, whence an individual who has left a mark may be surely identified. All this he did, and the method is now in successful use in the criminal departments of every civilised country.

It is due to Galton, far more than to any other man, that many attributes of man, which at first sight appear only susceptible of qualitative estimation, have been made reducible to exact measurement. Some people have thought that some of his ideas were elaborate jokes, and, indeed, he himself enjoyed the humorous side of his attempts as much as anyone. But such a view would be quite erroneous, for it will be perceived on closer scrutiny that he was always trying—and generally successfully—to measure something which might, perhaps, be regarded as beyond the scope of an exact estimate. Measurement is the soul of science, and he was thus carrying the accuracy of scientific investigation into new fields. Thus he made a beauty-map of England and Scotland, showing the geographical distribution of good looks in the population, and he devised the method of composite photographs, in which each member of a group of persons made an equal impress on the resulting portrait. In this way family or other resemblances were given concrete shapes. He tried also to register the individualities of faces, while annulling their common features, but the attempt did not lead to any intelligible conclusions and was a failure.

Galton also made important and very original contributions to Psychology. It was thought by earlier investigators that if they could discover by introspection how their own minds worked, they would have solved the general problem of the working of the human intellect. But Galton showed that different minds work in different ways, and, for example, that visual images play a large part with many people, but not so with others. In this connection he investigated the pictures of scenes recalled in memory, as to illumination, definition, colouring, and as to other peculiarities. Akin to this was an inquiry into visions seen by the sane, which he found to be much more frequent and realistic than is generally supposed to be the case. A curious example, of a somewhat analogous character, is afforded by the visual patterns or pictures associated in many minds with numbers. He also experimented on the senses of taste and smell, on the power of accurately estimating weight by the muscular sense, on the judgment of experts in guessing the weights of cattle, and on other such matters too numerous to mention. This mere catalogue of highly original investigations, and the fact that he was the first man in England to make psychometric experiments and to publish the results, show that Galton deserves a high rank amongst experimental psychologists, and yet his investigations were merely collateral to the main line of his work.

When in 1859 the 'Origin of Species' was published by his cousin, Charles Darwin, Galton became at once a convert, and began to reflect deeply on the problems of inheritance, especially as applicable to the human race. He was impressed by the fact that many of those who obtained distinction in the University at Cambridge were related to others who had been similarly distinguished at earlier dates. He therefore made a series of statistical inquiries as to the heritability of genius of all kinds. From first to last these investigations extend over a period of nearly forty years, and are to be found embodied in his works: 'Hereditary Genius,' 1869; 'English Men of Science,' 1874; 'Human Faculty,' 1883; 'Natural Inheritance,' 1889; and 'Noteworthy Families,' 1906. These works establish beyond any doubt the inheritance of mental capacity, as well as of all other physical characteristics.

Such investigations as these necessarily brought before him the fundamental principles of statistics, and although his mathematical equipment was insufficient to enable him to treat his many problems with completeness, yet his grasp of principles enabled him to obtain a remarkably clear insight into that difficult subject. In the hands of Karl Pearson and of others, the impulse given by Galton has led to the formulation of new statistical methods, of which much use has been made in the study of heredity. It would be out of place, in the present article, to give even an outline of such a technical subject, and it must suffice to say that it is now possible to assign a numerical value for the average degree of relationship or "correlation" between any pair of attributes in a large population. In close relationship to the theory of correlation is Galton's conclusion that the *average* contribution to each individual is $\frac{1}{4}$ from each parent, $\frac{1}{16}$ from each grand-parent, and so on for the remoter generations. This conclusion remains but little shaken by the copious criticisms to which it has been subjected by many other investigators.

It may be well to mention, in passing, that Galton made some interesting experiments on the breeding of rabbits, with a view of testing Darwin's theory of pangenesis. He argued that a copious transfusion of blood between two individuals of different varieties should carry with it some of the reproductive "gemmules," and that the offspring should show some of the characteristics of the variety whose blood had tainted the parents. But the result was negative, for no effect could be traced.

The conviction that all attributes are heritable naturally led Galton to reflect on the improvement of the human race which might be effected by breeding from the best and restricting the offspring of the worst. He gave the name of Eugenics to this branch of study, and it is probable that it is through Eugenics that he will always be best known to the larger public which cares little for science, but will attend to matters touching every member of the human race. Careful breeding might produce results as remarkable in mankind as it has done with domestic animals, but Galton was under no illusion as to the rapidity with which favourable results will be attained. He foresaw that, in the present condition of society, immediate measures were impracticable, except perhaps in restraints to the breeding from idiots and the feeble-minded, and he thought that education in a knowledge of the power of heredity would take several generations to permeate through all ranks of the community. Eugenics Societies have already been founded, and such considerable progress has been made that Galton's expectations may well prove to have been too pessimistic.

With the object of promoting investigation Galton initiated a Eugenics Office in 1905, and this led to the foundation of a Eugenics Laboratory in 1906 to be worked by Karl Pearson in connection with his Biometric Laboratory already referred to above. He further endowed a Research Fellowship and Scholarship in connection with these institutions. A quarterly journal, entitled 'Biometrika,' for the publication of researches had already been founded in 1901, and Galton was asked to be Consulting Editor.

He said of himself that he took "Eugenics very seriously, feeling that its principles ought to become one of the dominant motives in a civilised nation, much as if they were one of its religious tenets."* It has been shown that during his life he was the driving force of the movement, not only by his writings, but also by his endowment of research in this field. And after his death it was found that, subject to certain specific bequests, he had left his residual estate, amounting to about £45,000, for the foundation of a Chair of Eugenics in the University of London, with the expressed wish that Karl Pearson should become the first Professor, a wish which has since been fulfilled. The capital sum was as far as possible to be left intact for the maintenance of the Chair, and the necessary laboratory was to be provided in some other way. Since his death a subscription has been initiated for the latter purpose.

This large endowment will be of enormous benefit to the cause which Galton had so much at heart, and if his forecast of the future shall be fulfilled, he will rank not merely as a great investigator, but also as amongst the greatest of benefactors to mankind.

G. H. D.

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* 'Memories of my Life,' p. 322.

## JOHN HUGHLINGS JACKSON, 1835—1911.

John Hughlings Jackson, whose death occurred on October 7, 1911, at the age of 76, had been a Fellow of the Society since 1878. By his death, English medicine, and neuro-pathology in particular, has lost one of its most original and illustrious exponents.

Hughlings Jackson was born in 1835 of a Yorkshire father and a Welsh mother, in the village of Green Hammerton, near Knaresborough, in the county of York. His early education was entirely provincial. He acquired a fair knowledge of French, but he never learnt German, and often lamented his inability to read treatises in this language at first hand.

As was the fashion in those days, he began his medical studies by becoming apprenticed to a practitioner—Dr. Anderson, of York—and attended lectures at the York Hospital Medical School, a small and unimportant institution. At this institution Sir Jonathan Hutchinson, Jackson's lifelong friend, also commenced his medical studies.

In 1855 Jackson entered St. Bartholomew's Hospital, where he became a pupil of Sir James Paget, then in the height of his fame as a clinical teacher. After six months' study at St. Bartholomew's, he passed his examinations for the qualifications of M.R.C.S. and L.S.A., and returned to York, where he was appointed House Surgeon to the York Dispensary, a post which he held for two years. It was during this time that he came under the influence of Dr. Thomas Laycock, afterwards Professor of Medicine in the University of Edinburgh.

Laycock was a man of extraordinary suggestiveness and almost prophetic insight. He and Jackson had many points in common, though in accuracy of clinical observation Jackson far surpassed him. But, like many other of his pupils, Jackson always freely acknowledged his great indebtedness to Laycock's brilliant and stimulating speculations.

In 1859 Jackson came to London with a recommendation to Sir Jonathan Hutchinson, who introduced him to London hospital work, and helped him much in his early career. Hutchinson has always properly taken credit for having "discovered" Jackson, and for having dissuaded him from giving up medicine, as he at one time seemed inclined to do ("The Late Dr. Hughlings Jackson: Recollections of a Lifelong Friendship," 'Brit. Med. Journ.,' December 9, 1911, by Sir Jonathan Hutchinson).

In 1860 he took his degree of M.D. at St. Andrews, and was admitted as a member of the College of Physicians in the following year. In 1864 he was appointed Assistant Physician at the London Hospital and Lecturer on Physiology at its Medical School. He was appointed full physician in 1874, and held the post till 1894, when he was placed on the Consulting Staff. Concomitantly with his duties at the London Hospital, Jackson also acted as Assistant Physician (1863), and ultimately (1867) as Physician, to the

National Hospital for the Paralysed and Epileptic till 1906, when he retired from the active staff as Consulting Physician. At the National Hospital in particular, Jackson found a rich field for his neurological studies, towards which he was largely directed by the personal influence of Brown-Séquard.

During his earlier years he spent much time in reporting for the medical journals cases of interest in the various metropolitan hospitals, and made the acquaintance of the members of the staff of most of these institutions.

Throughout the whole of his career as Physician to the London and National Hospitals Jackson was busy with his pen, and his contributions to the medical journals, lectures, etc., had amounted in 1902 to over 200 (*vide* Bibliography appended to Sir W. Broadbent's Hughlings Jackson Lecture, 'Brain,' vol. 26, 1903, p. 356, *et seq.*). Though frequently urged by his friends to publish in a collected form his numerous contributions to medical science, scattered in various journals, and practically inaccessible to the great majority of students, he always made some excuse, and would not allow anyone to edit them in case of any inaccuracy or misrepresentation, of which he had a horror.

His voluminous writings embrace clinical observations, biological and philosophical speculations. In the latter the influence of Herbert Spencer, of whom he was an intimate friend and admirer, is largely seen. There is much repetition and iteration of the dominant ideas which form the groundwork of his teaching.

His style is frequently obscure, owing to the numerous provisos and qualifications which he constantly introduced to prevent his being misunderstood. But a noteworthy feature in his writings is that he never failed to indicate any facts which seemed to contradict his own theories or explanations.

One of his earliest services to clinical medicine, and clinical neurology in particular, was his demonstration that optic neuritis in cerebral disease may be consistent with the most perfect vision. He strongly urged the routine use of the ophthalmoscope in medicine, pointing out its incalculable importance in diagnosis. Indeed, this cannot be over-estimated, for without the ophthalmoscope the neuro-pathologist would be deprived of his most potent instrument of investigation.

It is, however, with his studies of convulsions and his views on the evolution and dissolution of the nervous system that his name is best known and most firmly associated.

When Jackson began his clinical work, the views of Flourens on the unity and indivisibility of the cerebral hemispheres were prevalent in the schools. About the time (1861) when Broca had established the probable relationship between aphasia and lesion of the third frontal convolution of the left hemisphere, Jackson had already observed the relatively frequent association of loss of speech with right hemiplegia, and in 1864 he had already seen seventy such cases.

His observations of cases of unilateral right-sided convulsions, followed by temporary loss of power and loss of speech, led him to conclude that these were the counterpart of hemiplegia, and dependent, not on destruction, but discharging lesion, followed by exhaustion of the same region. "From the point of view of function there are two ways in which nerve tissue suffers. It may be destroyed, and then there is loss of function. It may be unstable, and then there is disorder of function-discharge. In the case of nervous organs representing movements, we have palsy from destruction, and we have irregular movements (chorea), occasional spasm, etc., from instability" ("A Study of Convulsions," 'Trans. Med. Grad. Assoc.,' vol. 3, 1870). The region affected he described vaguely as the convolutions related to the corpus striatum, the region supplied by the Sylvian artery. In reply to possible objections on the ground that the cerebral hemispheres were the organ of the mind, he remarks:—

"It is asserted by some that the cerebrum is the organ of mind, and that it is not a motor organ. Some think the cerebrum is to be likened to an instrumentalist, and the motor centres to the instrument; one part is for ideas, and the other for movements. It may then be asked, How can discharge of part of a mental organ produce motor symptoms only? I say motor symptoms only, because, to give sharpness to the argument, I will suppose a case in which there is unilateral spasm without loss of consciousness. But of what 'substance' can the organ of mind be composed, unless of processes representing movements and impressions; and how can the convolutions differ from the inferior centres, except as parts representing more intricate co-ordinations of impressions and movements in time and space than they do? Are we to believe that the hemisphere is built on a plan fundamentally different from that of the motor tract? What can an 'idea' (say, of a ball) be except a process representing certain impressions of surface and particular muscular adjustments? What is recollection but a revivification of such processes which, in the past, have become part of the organism itself? What is delirium, except the disorderly revival of sensori-motor processes received in the past? What is a mistake in a word, but a wrong movement, a chorea? Giddiness can be but the temporary loss or disorder of certain relations in space, chiefly made up of muscular feelings. Surely the conclusion is irresistible, that 'mental' symptoms from disease of the hemisphere are fundamentally like hemiplegia, chorea, and convulsions, however specially different. They must all be due to lack, or to disorderly development, of sensori-motor processes" ('Trans. St. And. Med. Grad. Assoc.,' vol. 3, 1870).

Jackson's views as to the constitution of the cerebral hemispheres and the existence of motor centres for the limbs, face, etc., in the Rolandic area were confirmed by Hitzig (1870) and subsequent experimenters. By his own careful observation of the onset, limitation and march of the spasms in cases of disease, he himself largely contributed to the exact localisation in man of the various motor centres experimentally determined on the lower animals. He, however, never accepted the doctrine of exclusive localisation, holding that though each centre represents one set of movements in particular, yet it represents all more or less.

In the phenomena of disease Jackson always insisted on there being a positive as well as a negative element. This is the central idea of his explanation of the phenomena of insanity, post-epileptiform states, aphasia, etc., and is founded on his views as to the evolution of the nervous system.

These cannot be better given than in his own words:—

"Beginning with evolution, and dealing only with the most conspicuous parts of the process, I say of it that it is an ascending development in a particular order. I make three statements which, although from different standpoints, are about the very same thing. (1) Evolution is a passage from the most to the least organised, that is to say, from the lowest, well organised, centres up to the highest, least organised, centres; putting this otherwise, the progress is from centres comparatively well organised at birth up to those, the highest centres, which are continually organising through life. (2) Evolution is a passage from the most simple to the most complex; again, from the lowest to the highest centres. There is no inconsistency whatever in speaking of centres being at the same time most complex and least organised. Suppose a centre to consist of but two sensory and motor elements; if the sensory and motor elements be well joined, so that 'currents flow' easily from the sensory into the motor elements, then that centre, although a very simple one, is highly organised. On the other hand, we can conceive a centre consisting of four sensory and four motor elements, in which, however, the junctions between the sensory and motor elements are so imperfect that the nerve currents meet with much resistance. Here is a centre twice as complex as the one previously spoken of, but of which we may say that it is only half as well organised. (3) Evolution is a passage from the most automatic to the most voluntary.

"The triple conclusion come to is that the highest centres, which are the climax of nervous evolution, and which make up the 'organ of mind' (or physical basis of consciousness), are the least organised, the most complex, and the most voluntary. So much for the positive process by which the nervous system is 'put together'—evolution. Now for the negative process, the 'taking to pieces'—dissolution.

"Dissolution being the reverse of the process of evolution just spoken of, little need be said about it here. It is a process of undevelopment; it is a 'taking to pieces' in the order from the least organised, from the most complex and most voluntary, towards the most organised, most simple, and most automatic. I have just used the word 'towards,' for if dissolution were up to and inclusive of the most organised, etc., if, in other words, dissolution were total, the result would be death. I say nothing of total dissolution in these lectures. Dissolution being partial, the condition in every case of it is duplex. The symptomatology of nervous diseases is a double condition; there is a negative and there is a positive element in every case. Evolution not being entirely reversed, some level of evolution is left. Hence the statement, 'to undergo dissolution,' is rigidly the equivalent of the statement, 'to be reduced to a lower level of evolution.' In more detail, loss of the least organised, most complex, and most voluntary, implies the retention of the more organised, the less complex, and the more automatic. This is not a mere truism, or, if it be, it is one that is often neglected. Disease is said to 'cause' the symptoms of insanity. I submit that disease only produces negative mental symptoms answering to the dissolution, and that all elaborate positive mental symptoms (illusions, hallucinations, delusions, and extravagant conduct) are the outcome of activity of nervous elements untouched by any pathological process; that they arise during activity on the lower level of evolution remaining" (Croonian Lectures "On Evolution and Dissolution of the Nervous System," 1884, 'Brit. Med. Journ.,' 1, 1884).

The three "levels" of evolution are thus described :—

"I will state what I believe to be the hierarchy of nervous centres, which accords with the doctrine of evolution. I used to arrange them according to the morphological divisions of the nervous system—spinal cord, medulla oblongata, etc. I now arrange them on an anatomico-physiological basis, that is, especially as to degree of indirectness with which each represents the body, or part of it. The lowest motor centres are the anterior horns of the spinal cord, and also the homologous nuclei for motor cranial nerves higher up; they extend from the lowest spinal anterior horns up to the nuclei for the ocular muscles. They are at once lowest cerebral and lowest cerebellar centres; hence lesion of them cuts off the parts they represent from the whole central nervous system. I am ignoring the cerebellar system (see *infra*, p. 6). The lowest centres are the most simple and the most organised centres; each represents some limited region of the body indirectly, but yet most nearly directly; they are representative. The middle motor centres are the convolutions making up Ferrier's motor region. These are more complex and less organised, and represent wider regions of the body doubly indirectly; they are re-representative. The highest motor centres are convolutions in front of the so-called motor region. I say 'so-called,' as I believe, and have urged for many years, that the whole anterior part of the brain is motor, or chiefly motor. I speak more in detail of this in another lecture. The highest motor centres are the most complex and least organised centres, and represent widest regions (movements of all parts of the body) triply indirectly; they are re-re-representative. That the middle motor centres represent over again what all the lowest motor centres have represented, will be disputed by few. I go further, and say that the highest motor centres (frontal lobes) represent over again, in more complex combinations, what the middle motor centres represent. In recapitulation, there is increasing complexity, or greater intricacy of representation, so that ultimately the highest motor centres represent, or, in other words, co-ordinate, movements of all parts of the body in the most special and complex combinations. It is needless to give the scheme of sensory centres. The main conclusions are (1) that the highest (chiefly) sensory centres—parts behind Ferrier's sensory region—and also the highest (chiefly) motor centres—parts in front of the so-called motor region—make up the physical basis of consciousness; and (2) that just as consciousness represents, or is, the whole person psychical, so its basis (highest centres) represents the whole person physical—represents impressions and movements of all parts of his body, in old-fashioned language, the highest centres are potentially the whole organism. States of consciousness attend survivals of the fittest states of centres representing the whole organism" (*Ibid.*).

As to his highest levels and their situation in the brain his views do not claim to be more than speculations, and much will have to be done before they can be accepted as of higher value.

It is of interest that his views as to the function and mode of action of the cerebellum have been in all essentials confirmed by recent experimental research. He says:—

"All the muscles of the body are innervated both by the cerebrum and cerebellum, but in an inverse order. The cerebellum regulates the muscular contractions necessary for our attitudes in space, while the cerebrum regulates the contractions necessary to effect all changes of attitude which are made in response to successive impressions occurring in time. Speaking broadly, then, the cerebellum regulates continuous or tonic muscular contractions. It will be seen, therefore, that every combined muscular adjustment necessitates the co-operation of both these organs; no change of attitude can be effected by the cerebrum except in so far as a certain attitude was previously maintained by the cerebellum, and no steady movements can be produced by the alternate contractions of some groups of muscles, except in so far as other groups of muscles are maintained in a state of continuous contraction. Hence it may be inferred that all movements of the body are co-ordinated both in the cerebellum and the cerebrum."

He ingeniously explained many of the phenomena of disease associated with rigidity or contracture, such as paralysis agitans, hemiplegic and paraplegic contracture, by unantagonised cerebellar influx, owing to cessation or diminution of the influence of the cerebral hemispheres.

The above extracts convey only a meagre sketch of the chief fundamental principles which he applied to the elucidation of the phenomena of disease with so much originality and fruitfulness.

Jackson was a bad teacher in the ordinary sense, and lectured over the heads of the rank and file of his students. Yet, in spite of all this, there was never any unseemly behaviour in his class, such as occurred in that of some of his colleagues, or wherever a teacher is not *en rapport* with his pupils. Though he was essentially unpractical in a worldly sense, no one took liberties with him, and he enjoyed the reputation of being a genius, and on a higher level than ordinary men. He was not only revered, but beloved by all with whom he came in contact. He was utterly devoid of self-seeking.

In argument he was as courteous and considerate to the merest tyro as to the most eminent of his professional colleagues. He was of a shy, retiring disposition, grave, and in appearance much older than his years, and was familiarly known to his colleagues as "the Sage of Manchester Square." Sitting absorbed in thought in the corner of his landau, as he drove about on his professional rounds, he was a familiar figure in the West End.

Though serious in aspect, he had a fund of dry humour, and enjoyed a joke, even at his own expense. He was easily bored, and would take a play at the theatre in two or more instalments, necessitating separate tickets, rather than sit out the whole at once. When dining with his friends, which he seldom did, he would not unfrequently get up, beg to be excused when a certain hour came, at whatever stage of the proceedings. He was not fond of foreign travel, but liked to take holidays driving about the country in his carriage. He had little or no artistic perception, and this, as Dr. Buzzard has remarked ('Brit. Med. Journ.,' Oct. 14, 1911), probably acted prejudicially on his style of composition.

He had no recreations beyond novel reading, which he indulged in to a large extent. Increasing deafness in the later years of his life caused him to keep aloof from scientific meetings and from society in general, so that he became more and more of a recluse.

Childless himself, he was passionately fond of children, and delighted to bring toys to the children of his colleagues, who all loved and trusted him with their confidences. He married his first cousin, to whom he was devotedly attached, and her death, over thirty years before his own, was an irreparable loss to him.

Besides a world-wide reputation among his professional brethren, Jackson received many honours and marks of affectionate esteem from his colleagues and pupils. He was elected to the Fellowship of the Royal Society in 1878. He was F.R.C.P. (Lond.) and Hon. F.R.C.P.I., LL.D. Edinburgh and Glasgow, D.Sc. of Leeds, and Hon. M.D. of the University of Bologna, an honour from abroad which gave him special pleasure. He delivered in succession the Gulstonian (1868), Croonian (1884), and Lumleian (1890) Lectures to the Royal College of Physicians.

The Neurological Society, of which he was the first President, founded the Hughlings Jackson Lectureship in his honour, and he delivered the first lecture of the series himself in 1897 on "The Relations of Different Divisions of the Cerebral Nervous System to One Another and to Parts of the Body." The second lecture was delivered in 1900 by Prof. Hitzig, on "Hughlings Jackson and the Cortical Motor Centres, in the Light of Physiological Research" ('Brain,' vol. 23, 1900).

When he retired from the active staff of the London Hospital, he was presented with his portrait (Calkin) by his colleagues and admirers at home and abroad "in recognition of their esteem and admiration of his great services to the London Hospital Medical College, his distinguished position in the profession, and the advances he effected in medical science by his laborious investigations and profound insight into diseases of the nervous system." This portrait is now in the possession of the Royal College of Physicians.

A marble bust (an excellent likeness by H. Hampton), subscribed for by his colleagues, graces the Entrance Hall of the National Hospital for the Paralysed and Epileptic, and reminds all who visit that institution of the great master who has passed away, but whose name will for ever remain enshrined in the annals of medical science.

D. F.

## JOHN BEDDOE, 1826—1911.

Dr. John Beddoe was born at Bewdley in West Worcestershire on September 21, 1826, and belonged to an old yeoman stock in South Shropshire. He was a quiet, sickly child and his parents very wisely did not allow him to be taught to read or write, but these accomplishments he picked up for himself about his eighth year. All through his early life he was subject to attacks of illness which threw him back in his studies. As a boy he showed an interest in geography, and was greedy of knowledge and not without originality. Dr. Beddoe had a peculiarly observant mind and always endeavoured to account for what he saw; this was characteristic of him from his youth, and his mental alertness and sympathy for new ideas continued with him to the end of his long life. He graduated in medicine in Edinburgh and London, and during this period came into personal contact with a number of men already distinguished or who were to become so, many of whom made a lasting impression on the friendly and sympathetic student. His first paper, 'A Contribution to Scottish Anthropology,' was published in 1853. A year or so later he volunteered to join the Civil Hospital Staff, then being formed to supplement the undermanned Army Medical Service, which could not overtake its work at that stage of the Crimean War. In the course of his medical duties and during the little trips that he made he came into relation with various races and peoples of Eastern Europe and Western Asia, the characteristics of which he duly noted. In 1856-7 he travelled through a great part of Europe, gaining anthropological experience all the while. In 1857 Dr. Beddoe settled down to medical practice in Bristol, from which he retired in 1891. During all these years he led the quiet, busy life of a medical practitioner, winning the affection and esteem of a wide circle of friends and fellow-townsmen. The monotony was broken by a few visits to the Continent and one to Australia. In 1910 he published a delightful and informing autobiography, entitled 'Memories of Eighty Years,' which should be read by every anthropologist as it throws many sidelights on the founders of the science and incidents in its history. Dr. Beddoe died on July 19, 1911, in the historic old house called the Chantry, at Bradford-on-Avon, where he had resided for ten years.

Dr. Beddoe's life roughly corresponds with the modern development of anthropology, and naturally he came into personal contact or entered into correspondence with most of those whose names are held in honour by students. The majority of anthropologists were then measuring skulls and exercising their ingenuity in devising new chords, arcs and angles, and the instruments wherewith to measure them, the heads of living individuals of diverse races being treated as far as possible in a similar manner. The shrewd Bristol doctor, who early in his medical career had applied his clinical training to the observation of the living, had stored his memory and note-books with observations of the physical and psychical characteristics of various races and peoples; though he made various investigations in craniology and osteology, mainly of the old inhabitants of these islands, his chief claim to fame will be as the pioneer and chief exponent of what may be termed "observational anthropology." It was he who first made statistical investigations upon the colour of the hair and eyes of European peoples. Owing to the observations of numerous Continental anthropologists on large numbers of conscripts and other groups of people we now have very definite information concerning the pigmentation and other characters of several European countries. Dr. Beddoe's data were compiled partly from statistics obtained from the 'Hue and Cry,' referring mainly to deserters from the army, and partly from his own observations, for the making of which he devised a very simple method. The main results of his investigations on the physical characters of the British people will be found in 'The Races of Britain: a Contribution to the Anthropology of Western Europe,' 1885, which still remains the only monograph on the subject. The book is an expansion of the memoir on 'The Origin of the English Nation,' for which he won in 1867 the prize of 100 guineas offered by the Council of the Welsh National Eisteddfod for the best essay on that subject. In 1891 Dr. Beddoe delivered the Rhind Lectures in Edinburgh, taking as his subject 'The Anthropological History of Europe.' They were published in 1893 in a small volume which cannot now be obtained. The treatment of the subject was less detailed and statistical than that of 'The Races of Britain,' but it constituted a valuable sketch of the physical anthropology of Europe, indeed it remained for several years the only one in the English language. A bibliography of Dr. Beddoe's papers and memoirs will be found in 'Man,' October, 1911, p. 152.

John Beddoe, M.D., LL.D., F.R.S., F.R.C.P.L., was Honorary Professor of Anthropology in the University of Bristol; Officier de l'Instruction Publique

(1re Classe); Vice-President and ex-President of the Royal Anthropological Institute; President of the Wiltshire Archæological and Natural History Society; Foreign Associate of the Anthropological Society, Paris; Corresponding Member of the Anthropological Societies of Berlin, Sweden, and Rome; Honorary Member of the Anthropological Societies of Brussels and Washington, and of the Imperial Society of Friends of Science, Moscow, etc.

A. C. H.

# INDEX TO VOL. LXXXIV. (B)

Acetylmethylcarbinol, bacterial production of (Harden and Norris), 492.
Acquired characters, investigation of instance of transmission of (Brown), 555.
Address of the President (Geikie), 435.
Adsorption as preliminary to chemical reaction (Bayliss), 81.
Agglutination, factors concerned in (Dean), 416.
Alcyonaria, type of new family of (Hickson), 195.
Amakebe, transmission by *Rhipicephalus* (Theiler), 112.
Antelope infected with *Trypanosoma gambiense* (Fraser and Duke), 484.
Arber (E. A. N.) On the Fossil Flora of the Forest of Dean Coalfield and the Relationships of the Coalfields of the West of England and South Wales, 543.
Armstrong (H. E. and E. F.) The Origin of Osmotic Effects. IV.—Note on the Differential Septa in Plants with reference to the Translocation of Nutritive Materials, 226; —— and Horton (E.) Herbage Studies. I.—*Lotus corniculatus*, a Cyanophoric Plant, 471.
*Astrosclera willeyana*, Lister, note on (Kirkpatrick), 579.

*Bacillus cloacæ*, chemical action of, on glucose, etc. (Thompson), 500.
Bacteria, influence of ionised air on (Thornton), 280.
Bacterial and other cells, method of disintegrating (Barnard and Hewlett), 57.
Barnard (J. E.) and Hewlett (R. T.) On a Method of Disintegrating Bacterial and other Organic Cells, 57.
Barratt (J. O. W.) Fractional Withdrawal of Complement and Amboceptor by Means of Antigen (Preliminary Note), 277.
Bateson (W.) See Vilmorin and Bateson; —— and Punnett (R. C.) On the Inter-relations of Genetic Factors, 3.
Bayliss (W. M.) The Properties of Colloidal Systems. II.—On Adsorption as Preliminary to Chemical Reaction, 81; —— III.—The Osmotic Pressure of Electrolytically Dissociated Colloids, 229.
Beddoe (J.) Obituary notice of, xxv.
Blood, action of radium on constituents of (Chambers and Russ), 124; —— new method for total volume of (Todd and White), 255.
Blood corpuscles, fate of red, injected into circulation, etc. (Todd and White), 255.
Blood volume of mammals and relation to surface area of body (Dreyer and Ray), 460.
Bottomley (W. B.) The Structure and Physiological Significance of the Root-nodules of *Myrica gale*, 215.
Boyce (Sir R.) Obituary notice of, iii.
Brain, motor localisation in gibbon's (Mott, Schuster, and Sherrington), 67.
Brown (T. G.) The Intrinsic Factors in the Act of Progression in the Mammal, 308; —— An Alleged Specific Instance of the Transmission of Acquired Characters.—Investigation and Criticism, 555.
Bruce (Sir D.) The Morphology of *Trypanosoma evansi* (Steel), 181; —— The Morphology of *Trypanosoma gambiense* (Dutton), 327.
Buchanan (G.) Note on Developmental Forms of *Trypanosoma brucei* (*pecaudi*) in the Internal Organs, etc., of the Gerbil (*Gerbillus pygargus*), 161.
Buckmaster (G. A.) and Gardner (J. A.) Ventilation of the Lung during Chloroform Narcosis, 347.

Cancerous ancestry and cancer incidence in mice (Murray), 42.

Carbon assimilation, mechanism of (Usher and Priestley), 101.

Carcinoma, viability of human, in animals (Williams), 191.

Caulfeild (A. H.) Factors in the Interpretation of the Inhibitive and Fixation Serum Reactions in Pulmonary Tuberculosis, 373; —— Preliminary Report upon the Injection of Rabbits with Protein-free (Tuberculo-) Antigen and Antigen-Serum Mixtures, 390.

Ceratopora, a new family of Alcyonaria (Hickson), 195.

Chambers (H.) and Russ (S.) The Action of Radium Radiations upon some of the Main Constituents of Normal Blood, 124.

Chloroform narcosis, ventilation of lung during (Buckmaster and Gardner), 347.

Cholesterol, origin and destiny of. VIII.—In liver of rabbits under various diets (Ellis and Gardner), 461.

Circulatory apparatus, distribution of soluble substances in frogs deprived of (Meltzer), 98.

Coalfield, fossil flora of Forest of Dean, and relation of West of England and South Wales fields (Arber), 543.

Colloidal systems, properties of (Bayliss), 81.

Colour contrast, simultaneous (Edridge-Green), 546; —— discrimination of (Edridge-Green), 116.

Complement and amboceptor, withdrawal of, by means of antigen (Barratt), 277.

Cousin marriage, inbreeding in a Mendelian population with special reference to (Jacob), 23.

Cushny (A. R.) On the Action of Senecio Alkaloids and the Causation of Hepatic Cirrhosis in Cattle (Preliminary Note), 188.

Darwin (F.) and Pertz (D. F. M.) On a New Method of Estimating the Aperture of Stomata, 136.

Dean (H. R.) On the Factors concerned in Agglutination, 416.

Douglas (C. G.) and Haldane (J. S.) The Causes of Absorption of Oxygen by the Lungs in Man (Preliminary Communication), 1.

Dreyer (G.) and Ray (W.) Further Experiments upon the Blood Volume of Mammals and its Relation to the Surface Area of the Body, 460.

Duke (H. L.) See Fraser and Duke.

*Echinus* and *Echinocardium*, effects of crossing (MacBride), 394.

Edridge-Green (F. W.) The Discrimination of Colour, 116; —— Simultaneous Colour Contrast, 546.

Electric charges of living cells (Hardy and Harvey), 217.

Electrical effects accompanying decomposition (Potter), 260.

Ellis (G. W.) and Gardner (J. A.) The Origin and Destiny of Cholesterol in the Animal Organism. Part VIII.—On the Cholesterol Content of the Liver of Rabbits under Various Diets and during Inanition, 461.

Enteritis of bovines, preparation of diagnostic vaccine for pseudo-tuberculous (Twort and Ingram), 517.

Eye, sensibility to variations of wave-length (Watson), 118.

Eye media of Australian animals (Jona), 345.

Fantham (H. B.) *Herpetomonas pediculi*, nov. spec., parasitic in the Alimentary Tract of *Pediculus vestimenti*, the Human Body Louse, 505.

Flack (M.) See Hill and Flack.

Fraser (A. D.) and Duke (H. L.) Antelope infected with *Trypanosoma gambiense*, 484.

Fry (W. B.) A Preliminary Note on the Extrusion of Granules by Trypanosomes, 79.

Galton (Sir F.) Obituary notice of, x.

Gametic coupling in *Pisum* (Vilmorin and Bateson), 9; ——— and repulsion in *Primula* (Gregory), 12.

Gardner (J. A.) See Buckmaster and Gardner; Ellis and Gardner.

Geikie (Sir A.) Address at Anniversary Meeting, 1911, 435.

Genetic factors, inter-relations of (Bateson and Punnett), 3.

Goitre, experimental transmission from man to animals (McCarrison), 155.

Goodey (T.) A Contribution to our Knowledge of the Protozoa of the Soil, 165.

Gregory (R. P.) On Gametic Coupling and Repulsion in *Primula sinensis*, 12.

Guaiacum reaction given by plant extracts (Wheldale), 121.

Haldane (J. S.) See Douglas and Haldane.

Harden (A.) and Norris (D.) The Bacterial Production of Acetylmethylcarbinol and 2.3-Butylene Glycol from Various Substances, 492; —— and Paine (S. G.) Action of Dissolved Substances upon the Autofermentation of Yeast, 448.

Hardy (W. B.) and Harvey (H. W.) Note on the Surface Electric Charges of Living Cells, 217.

Harvey (H. W.) See Hardy and Harvey.

Hayden (A. F.) and Morgan (W. P.) An Inquiry into the Influence of the Constituents of a Bacterial Emulsion on the Opsonic Index, 320.

Hepatic cirrhosis in cattle, Senecio alkaloids and causation of (Cushny), 188.

Herbage studies. I.—*Lotus corniculatus* (Armstrong and Horton), 471.

Heredity, studies in (MacBride), 394.

*Herpetomonas pediculi*, n. sp., parasitic in alimentary tract of *Pediculus* (Fantham), 505.

Hewlett (R. T.) Immunisation by means of Bacterial Endotoxins, 49.

Hickson (S. J.) On Ceratopora, the Type of a New Family of Alcyonaria, 195.

Hill (L.) and Flack (M.) The Physiological Influence of Ozone, 404.

Horton (E.) See Armstrong and Horton.

Immunisation by bacterial endotoxins (Hewlett), 49.

Inbreeding in a Mendelian population, with reference to cousin marriage (Jacob), 23.

Infusorian micronucleus and regeneration (Lewin), 332.

Ingram (G. L. Y.) See Twort and Ingram.

Ionised air, influence on bacteria (Thornton), 280.

Jackson (J. H.) Obituary notice of, xviii.

Jacob (S. M.) Inbreeding in a Stable Simple Mendelian Population with Special Reference to Cousin Marriage, 23.

Jona (J. L.) The Refractive Indices of the Eye Media of some Australian Animals, 345.

Kennedy (R.) Experiments on Restoration of Paralysed Muscles by means of Nerve Anastomosis, 75.

Kirkpatrick (R.) Note on *Astrosclera willeyana*, Lister, 579.

Lewin (K. R.) The Behaviour of the Infusorian Micronucleus in Regeneration, 332.

*Lotus corniculatus*, a cyanophoric plant (Armstrong and Horton), 471.

Lungs, causes of absorption of oxygen by (Douglas and Haldane), 1.

MacBride (E. W.) Studies in Heredity. I.—The Effects of crossing the Sea-urchins *Echinus esculentus* and *Echinocardium cordatum*, 394.

McCarrison (R.) The Experimental Transmission of Goitre from Man to Animals, 155.

Mackenzie (K.) See Schäfer and Mackenzie.

Meltzer (S. J.) On the Distribution and Action of Soluble Substances in Frogs deprived of their Circulatory Apparatus, 98.

Milk secretion, action of animal extracts on (Schäfer and Mackenzie), 16.

Morgan (W. P.) See Hayden and Morgan.

Motor localisation in brain of gibbon (Mott, Schuster, and Sherrington), 67.

Mott (F. W.), Schuster (E.), and Sherrington (C. S.) Motor Localisation in the Brain of the Gibbon, correlated with a Histological Examination, 67.

Murray (J. A.) Cancerous Ancestry and the Incidence of Cancer in Mice, 42.

*Mycobacterium enteritidis*, &c., method of isolating and cultivating (Twort and Ingram). 517.

*Myrica gale*, structure, etc., of root-nodules of (Bottomley), 215.

Nerve anastomosis, restoration of paralysed muscles by (Kennedy), 75.

Norris (D.) See Harden and Norris.

Obituary Notices of Fellows Deceased :—

- Ringer (S.), i.
- Boyce (Sir R.), iii.
- Galton (Sir F.), x.
- Jackson (J. H.), xviii.
- Beddoe (J.), xxv.

Opsonic index, influence of constituents of bacterial emulsion on (Hayden and Morgan), 320.

Osmotic effects, origin of (Armstrong), 226.

Osmotic pressure of electrolytically dissociated colloids (Bayliss), 229.

Ozone, physiological influence of (Hill and Flack), 404.

Paine (S. G.) The Permeability of the Yeast-cell, 289 ; —— see also Harden and Paine.

Paralysed muscles, restoration by nerve anastomosis (Kennedy), 75.

Pertz (D. F. M.) See Darwin and Pertz.

Plants, differential septa in, and translocation of nutritive materials (Armstrong), 226.

Potter (M. C.) Electrical Effects accompanying the Decomposition of Organic Compounds, 260.

Priestley (J. H.) See Usher and Priestley.

Progression in mammal, intrinsic factors in act of (Brown), 308.

Protozoa of soil, contribution to knowledge of (Goodey), 165.

Pulmonary tuberculosis, interpretation of serum reactions in (Caulfeild), 373.

Punnett (R. C.) See Bateson and Punnett.

Radium radiations, action upon blood constituents (Chambers and Russ), 124.

Ray (W.) See Dreyer and Ray.

Reflex inhibition of knee flexor (Sherrington and Sowton), 201.

Ringer (S.). Obituary notice of, i.

Root-nodules of *Myrica gale*, structure, etc. (Bottomley), 215.

Russ (S.) See Chambers and Russ.

Schäfer (E. A.) and Mackenzie (K.) The Action of Animal Extracts on Milk Secretion, 16.

Schuster (E.) See Mott, Schuster, and Sherrington.

Senecio alkaloids and hepatic cirrhosis in cattle (Cushny), 188.

Serum reactions in pulmonary tuberculosis (Caulfeild), 373.

Sherrington (C. S.) See Mott, Schuster, and Sherrington ; —— and Sowton (S. C. M.) On Reflex Inhibition of the Knee Flexor, 201.

Soil, a contribution to knowledge of protozoa of (Goodey), 165.

Sowton (S. C. M.) See Sherrington and Sowton.

Stannus (H. S.) and Yorke (W.) The Pathogenic Agent in a Case of Human Trypanosomiasis in Nyasaland, 156.
Stomata, new method of estimating aperture of (Darwin and Pertz), 136.

Theiler (A.) Transmission of Amakebe by means of *Rhipicephalus appendiculatus*, the Brown Tick, 112.
Thompson (J.) The Chemical Action of *Bacillus cloacæ* (Jordan) on Glucose and Mannitol, 500.
Thornton (W. M.) The Influence of Ionised Air on Bacteria, 280.
Todd (C.) and White (R. G.) On the Fate of Red Blood Corpuscles when Injected into the Circulation of an Animal of the same Species; with a New Method for the Determination of the Total Volume of the Blood, 255.
*Trypanosoma brucei* (*pecaudi*), developmental forms of, in *Gerbillus pygargus* (Buchanan), 161; *T. evansi*, morphology of (Bruce), 181; *T. gambiense*, morphology of (Bruce), 327.
Trypanosomiasis in Nyasaland, pathogenic agent in (Stannus and Yorke), 156.
Tuberculo-antigen and antigen-serum mixtures, report on injection of rabbits with protein-free (Caulfeild), 390.
Twort (F. W.) and Ingram (G. L. Y.) A Method for Isolating and Cultivating the *Mycobacterium enteritidis chronicæ pseudotuberculosæ bovis*, Jöhne, and some Experiments on the Preparation of a Diagnostic Vaccine for Pseudo-tuberculous Enteritis of Bovines, 517.

Usher (F. L.) and Priestley (J. H.) The Mechanism of Carbon Assimilation. Part III. 101.

Vilmorin (P. de) and Bateson (W.) A Case of Gametic Coupling in Pisum, 9.

Watson (W.) Note on the Sensibility of the Eye to Variations of Wave-length, 118.
Wheldale (M.) On the Direct Guaiacum Reaction given by Plant Extracts, 121.
White (R. G.) See Todd and White.
Williams (C. L.) The Viability of Human Carcinoma in Animals, 191.

Yeast, action of dissolved substances on autofermentation of (Harden and Paine), 448.
Yeast-cell, permeability of (Paine), 289.

END OF THE EIGHTY-FOURTH VOLUME (SERIES B).

---

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# PROCEEDINGS
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# THE ROYAL SOCIETY.

## Series B. Vol. 84. No. B 568.

### BIOLOGICAL SCIENCES.

## CONTENTS.

JUL 18 1911

Page

The Causes of Absorption of Oxygen by the Lungs in Man. (Preliminary Communication.) By C. GORDON DOUGLAS, B.M., Fellow of St. John's College, and J. S. HALDANE, M.D., LL.D., F.R.S., Fellow of New College, Oxford . . . . . . . . . . . . . 1

On the Inter-relations of Genetic Factors. By W. BATESON, F.R.S., and R. C. PUNNETT, Professor of Biology in the University of Cambridge . 3

A Case of Gametic Coupling in Pisum. By PHILIPPE DE VILMORIN and W. BATESON, F.R.S. . . . . . . . . . . . 9

On Gametic Coupling and Repulsion in Primula sinensis. By R. P. GREGORY, M.A., Fellow of St. John's College, Cambridge, University Lecturer in Botany . . . . . . . . . . . . . 12

The Action of Animal Extracts on Milk Secretion. By E. A. SCHÄFER, F.R.S., and K. MACKENZIE, M.B., Ch.B. . . . . . . . . 16

Inbreeding in a Stable Simple Mendelian Population with Special Reference to Cousin Marriage. By S. M. JACOB, I.C.S., Biometric Laboratory, University College, London . . . . . . . . . 23

Cancerous Ancestry and the Incidence of Cancer in Mice. By J. A. MURRAY, M.D., B.Sc., Imperial Cancer Research Fund . . . . . . . 42

Immunisation by Means of Bacterial Endotoxins. By R. TANNER HEWLETT, M.D. . . . . . . . . . . . . . 49

On a Method of Disintegrating Bacterial and other Organic Cells. By J. E. BARNARD and R. T. HEWLETT. (Plate 1) . . . . . . 57

Motor Localisation in the Brain of the Gibbon, correlated with a Histological Examination. By F. W. MOTT, EDGAR SCHUSTER, and C. S. SHERRINGTON . . . . . . . . . . . . . 67

Experiments on the Restoration of Paralysed Muscles by means of Nerve Anastomosis. By ROBERT KENNEDY, M.A., M.D., D.Sc. (Abstract) . 75

A Preliminary Note on the Extrusion of Granules by Trypanosomes. By W. B. FRY, Captain R.A.M.C. . . . . . . . . . 79

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# PROCEEDINGS

# OF

# THE ROYAL SOCIETY.

**Series B. Vol. 84.** No. **B 569.**

**BIOLOGICAL SCIENCES.**

## CONTENTS.

Page

The Properties of Colloidal Systems.—II. On Adsorption as Preliminary to Chemical Reaction. By W. M. BAYLISS, F.R.S., Institute of Physiology, University College, London . . . . . . . . . . 81

On the Distribution and Action of Soluble Substances in Frogs Deprived of their Circulatory Apparatus. By S. J. MELTZER, New York . . . 98

The Mechanism of Carbon Assimilation : Part III. By FRANCIS L. USHER and J. H. PRIESTLEY . . . . . . . . . . 101

Transmission of Amakebe by means of Rhipicephalus appendiculatus, the Brown Tick. By Dr. A. THEILER, C.M.G., Pretoria . . . . . 112

The Discrimination of Colour. By F. W. EDRIDGE-GREEN, M.D., F.R.C.S., Beit Medical Research Fellow . . . . . . . . 116

Note on the Sensibility of the Eye to Variations of Wave-length. By W. WATSON, D.Sc., F.R.S. . . . . . . . . . . 118

On the Direct Guaiacum Reaction given by Plant Extracts. By Miss M. WHELDALE, Fellow of Newnham College, Cambridge . . . . 121

The Action of Radium Radiations upon some of the Main Constituents of Normal Blood. By HELEN CHAMBERS, M.D., and S. RUSS, D.Sc., Beit Memorial Research Fellow . . . . . . . . . . 124

On a New Method of Estimating the Aperture of Stomata. By FRANCIS DARWIN, F.R.S., and D. F. M. PERTZ . . . . . . . . . 136

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
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# PROCEEDINGS
OF
# THE ROYAL SOCIETY.

**Series B. Vol. 84.** **No. B 570.**

**BIOLOGICAL SCIENCES.**

## CONTENTS.

Page

The Experimental Transmission of Goitre from Man to Animals. By ROBERT McCARRISON, M.D., M.R.C.P. (Lond.), Captain, Indian Medical Service; Agency Surgeon, Gilgit, Kashmere. (Plate 1*) . . . . 155

The Pathogenic Agent in a Case of Human Trypanosomiasis in Nyasaland. By HUGH S. STANNUS and WARRINGTON YORKE. (Plate 2) . 156

Note on Developmental Forms of Trypanosoma brucei (pecaudi) in the Internal Organs, Axillary Glands and Bone-marrow of the Gerbil (Gerbillus pygargus). By GEORGE BUCHANAN, Senior Laboratory Assistant, Wellcome Tropical Research Laboratories, Gordon College, Khartoum. (Plate 3) . . . . . . . . . . . . 161

A Contribution to our Knowledge of the Protozoa of the Soil. By T. GOODEY, M.Sc. (Birm.), Mackinnon Student of the Royal Society, Rothamsted Experimental Station. (Plate 4) . . . . . . . 165

The Morphology of Trypanosoma evansi (Steel). By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service. (Plate 5) . . . . 181

On the Action of Senecio Alkaloids and the Causation of Hepatic Cirrhosis in Cattle. (Preliminary Note.) By ARTHUR R. CUSHNY, F.R.S. . 188

The Viability of Human Carcinoma in Animals. By Major C. L. WILLIAMS, M.D., I.M.S. (ret.) . . . . . . . . . . . . . 191

On Ceratopora, the Type of a New Family of Alcyonaria. By SYDNEY J. HICKSON, F.R.S., Professor of Zoology in the University of Manchester. (Plate 6) . . . . . . . . . . . . . . 195

On Reflex Inhibition of the Knee Flexor. By C. S. SHERRINGTON, F.R.S., and S. C. M. SOWTON. . . . . . . . . . . . . 201

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
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# PROCEEDINGS
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---

**Series B. Vol. 84. No. B 571.**

**BIOLOGICAL SCIENCES.**

---

## CONTENTS.

Page

The Structure and Physiological Significance of the Root-nodules of Myrica gale. By W. B. BOTTOMLEY, M.A., Professor of Botany in King's College, London . . . . . . . . . . . . . 215

Note on the Surface Electric Charges of Living Cells. By W. B. HARDY, F.R.S., and H. W. HARVEY . . . . . . . . . . . 217

The Origin of Osmotic Effects. IV.—Note on the Differential Septa in Plants with reference to the Translocation of Nutritive Materials. By HENRY E. ARMSTRONG, F.R.S., and E. FRANKLAND ARMSTRONG . 226

The Properties of Colloidal Systems. III.—The Osmotic Pressure of Electrolytically Dissociated Colloids. By W. M. BAYLISS, F.R.S., Institute of Physiology, University College, London . . . . . . . 229

On the Fate of Red Blood Corpuscles when Injected into the Circulation of an Animal of the same Species; with a New Method for the Determination of the Total Volume of the Blood. By CHARLES TODD, M.D., Bacteriologist, Egyptian Government; and R. G. WHITE, M.B., Director, Serum Institute, Cairo . . . . . . . . . . 255

Electrical Effects accompanying the Decomposition of Organic Compounds. By M. C. POTTER, Sc.D., M.A., Professor of Botany in the University of Durham . . . . . . . . . . . . . 260

Fractional Withdrawal of Complement and Amboceptor by Means of Antigen. (Preliminary Note.) By J. O. WAKELIN BARRATT, M.D., D.Sc. Lond., Director of Cancer Research Laboratory, University of Liverpool (Mrs. Sutton Timmis Memorial) . . . . . . . 277

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
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BIOLOGICAL SCIENCES.

## CONTENTS.

Page

The Influence of Ionised Air on Bacteria. By W. M. THORNTON, D.Sc., D.Eng., Professor of Electrical Engineering at Armstrong College, Newcastle-upon-Tyne. (Plates 7—12) . . . . . . . 280

The Permeability of the Yeast-Cell. By SYDNEY G. PAINE, Research Scholar in the Biochemical Department, Lister Institute, London . . 289

The Intrinsic Factors in the Act of Progression in the Mammal. By T. GRAHAM BROWN . . . . . . . . . . . 308

An Inquiry into the Influence of the Constituents of a Bacterial Emulsion on the Opsonic Index. By A. F. HAYDEN, M.B., B.S., F.R.C.S., I.M.S., and W. PARRY MORGAN, M.A., B.Sc., M.B. . . . . . . . 320

The Morphology of Trypanosoma gambiense (Dutton). By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S. (Plate 13) . . . . . . 327

The Behaviour of the Infusorian Micronucleus in Regeneration. By KENNETH R. LEWIN, B.A., Senior Scholar and Coutts Trotter Student of Trinity College, Cambridge . . . . . . . . . 332

The Refractive Indices of the Eye Media of some Australian Animals. By JUDAH LEON JONA, D.Sc., M.B., B.S. . . . . . . . 345

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**BIOLOGICAL SCIENCES.**

## CONTENTS.

Page

Ventilation of the Lung during Chloroform Narcosis. By G. A. BUCKMASTER and J. A. GARDNER . . . . . . . . . . . **347**

Factors in the Interpretation of the Inhibitive and Fixation Serum Reactions in Pulmonary Tuberculosis. By ALFRED H. CAULFEILD, M.B. . . **373**

Preliminary Report upon the Injection of Rabbits with Protein-free (Tuberculo-) Antigen and Antigen-Serum Mixtures. By ALFRED H. CAULFEILD, M.B. . . . . . . . . . . . . . **390**

Studies in Heredity. I.—The Effects of Crossing the Sea-urchins Echinus esculentus and Echinocardium cordatum. By Prof. E. W. MACBRIDE, F.R.S., Imperial College of Science and Technology, South Kensington . **394**

The Physiological Influence of Ozone. By LEONARD HILL, F.R.S., and MARTIN FLACK . . . . . . . . . . . . . **404**

On the Factors Concerned in Agglutination. By H. R. DEAN, M.A., M.B., Ch.B., M.R.C.P., Assistant Bacteriologist, Lister Institute, London . . **416**

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
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OF
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---

**Series B. Vol. 84. No. B 574.**

**BIOLOGICAL SCIENCES.**

---

## CONTENTS.

Page

Address of the President, Sir ARCHIBALD GEIKIE, K.C.B., at the Anniversary Meeting on November 30, 1911 . . . . . . . . . 435

Action of Dissolved Substances upon the Autofermentation of Yeast. By ARTHUR HARDEN, F.R.S., and SYDNEY G. PAINE . . . . . 448

Further Experiments upon the Blood Volume of Mammals and its Relation to the Surface Area of the Body. By GEORGES DREYER, M.A., M.D., Professor of Pathology in the University of Oxford, and WILLIAM RAY, M.B., B.Sc., Philip Walker Student in the University of Oxford (Abstract) . . . . . . . . . . . . . 460

The Origin and Destiny of Cholesterol in the Animal Organism. Part VIII.—On the Cholesterol Content of the Liver of Rabbits under Various Diets and during Inanition. By G. W. ELLIS and J. A. GARDNER . . . 461

Herbage Studies. I.—Lotus corniculatus, a Cyanophoric Plant. By HENRY E. ARMSTRONG, F.R.S., E. FRANKLAND ARMSTRONG and EDWARD HORTON . . . . . . . . . . . . 471

Antelope Infected with Trypanosoma gambiense. By Captain A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE . . . . . . . . . . 484

The Bacterial Production of Acetylmethylcarbinol and 2.3-Butylene Glycol from Various Substances. By ARTHUR HARDEN, F.R.S., and DOROTHY NORRIS . . . . . . . . . . . . 492

The Chemical Action of Bacillus cloacæ (Jordan) on Glucose and Mannitol. By JAMES THOMPSON . . . . . . . . . . . 500

Herpetomonas pediculi, nov. spec., Parasitic in the Alimentary Tract of Pediculus vestimenti, the Human Body Louse. By H. B. FANTHAM, D.Sc., B.A., Christ's College, Cambridge, and Liverpool School of Tropical Medicine (Plate 14) . . . . . . . . . . . 505

---

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---

**Series B. Vol. 84.** **No. B 575.**

**BIOLOGICAL SCIENCES.**

---

## CONTENTS.

Page

A Method for Isolating and Cultivating the Mycobacterium enteritidis chronicæ pseudotuberculosæ bovis, Jöhne, and Some Experiments on the Preparation of a Diagnostic Vaccine for Pseudo-tuberculous Enteritis of Bovines. By F. W. TWORT, M.R.C.S., L.R.C.P. Lond., and G. L. Y. INGRAM, M.R.C.V.S. . . . . . . . . . . . **517**

On the Fossil Flora of the Forest of Dean Coalfield (Gloucestershire), and the Relationships of the Coalfields of the West of England and South Wales. By E. A. NEWELL ARBER, M.A., F.L.S., F.G.S., Trinity College, Cambridge, University Demonstrator in Palæobotany. (Abstract) . . **543**

Simultaneous Colour Contrast. By F. W. EDRIDGE-GREEN, M.D., F.R.C.S., Beit Memorial Research Fellow . . . . . . . . . . **546**

An Alleged Specific Instance of the Transmission of Acquired Characters.—Investigation and Criticism. By T. GRAHAM BROWN (Carnegie Fellow) . **555**

Note on Astrosclera willeyana Lister. By R. KIRKPATRICK . . . **579**

Obituary Notices of Fellows Deceased:—Sydney Ringer (with portrait); Sir Rubert Boyce; Sir Francis Galton; John Hughlings Jackson; John Beddoe . . . . . . . . . . . . **i—xxvii**

Index . . . . . . . . . . . . . . **xxix**

Title, Contents, etc.

23055
MAR

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THE PATH OF AN ELECTRON IN COMBINED RADIAL MAGNETIC AND ELECTRIC FIELDS. By H. STANLEY ALLEN, M.A., D.Sc. .. 257

ON THE DYNAMICAL NATURE OF THE MOLECULAR SYSTEMS WHICH EMIT SPECTRA OF THE BANDED TYPE. By Prof. E. T. WHITTAKER, Sc.D., F.R.S. .. 262

ON SOME MINERAL CONSTITUENTS OF A DUSTY ATMOSPHERE. By W. N. HARTLEY, D.Sc., F.R.S. .. 271

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ON A METHOD OF MAKING VISIBLE THE PATHS OF IONISING PARTICLES THROUGH A GAS. By C. T. R. WILSON, M.A., F.R.S. (Plate 9) .. 285

THE VACUUM TUBE SPECTRA OF MERCURY. By FRANK HORTON, D.Sc., M.A. 288

THE SPECIFIC HEAT OF WATER. By W. R. BOUSFIELD, M.A., K.C., and W. ERIC BOUSFIELD, B.A. (Abstract) .. 302

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ON THE PROPAGATION OF WAVES THROUGH A STRATIFIED MEDIUM, WITH SPECIAL REFERENCE TO THE QUESTION OF REFLECTION. By LORD RAYLEIGH, O.M., F.R.S. .. .. .. .. .. .. .. .. .. .. .. .. .. .. .. .. 207

THE TESTING OF PLANE SURFACES. By P. E. SHAW, B.A., D.Sc. .. .. 227

NOTE ON THE SCATTERING OF α-PARTICLES. By Dr. H. GEIGER .. .. 235

THE EFFECT OF TEMPERATURE UPON RADIOACTIVE DISINTEGRATION. By ALEXANDER S. RUSSELL, M.A., B.Sc. .. .. .. .. .. .. .. .. .. .. .. 240

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